Basic Immunology for Vaccine

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Immunity

- **Innate (Natural) Immunity:**
  First line of defense, pattern recognition

- **Adaptive (Acquired) Immunity:**
  Need stimulation or infection, Memory and specific
Abbas et al, Cells and Molecular Immunology, 7 edition, 2012
Adaptive immunity against infections

Innate immunity

Humoral immunity vs. Cell-mediated immunity

- **Microbe**
  - Extracellular microbes
  - Phagocytosed microbes in macrophage
  - Intracellular microbes (e.g., viruses) replicating within infected cell

- **Responding lymphocytes**
  - B lymphocyte
  - Helper T lymphocyte
  - Cytotoxic T lymphocyte

- **Effector mechanism**
  - Secreted antibody

- **Transferred by**
  - Serum (antibodies)
  - Cells (T lymphocytes)

- **Functions**
  - Block infections and eliminate extracellular microbes
  - Activate macrophages to kill phagocytosed microbes
  - Kill infected cells and eliminate reservoirs of infection
Innate-adaptive Immunity

**Innate Immunity**

- **Specificity**: For structures shared by classes of microbes (pathogen-associated molecular patterns)
- **Receptors**: Encoded in germline; limited diversity (pattern recognition receptors)

**Adaptive Immunity**

- **Specificity**: For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens
- **Receptors**: Encoded by genes produced by somatic recombination of gene segments; greater diversity

*Abbas et al, Cells and Molecular Immunology, 7 edition, 2012*
### Ligands or microbes

**Table 4-2: Examples of PAMPs and DAMPs**

<table>
<thead>
<tr>
<th>Pathogen-Associated Molecular Patterns</th>
<th>Microbe Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleic acids</td>
<td>Virus, virus, bacteria</td>
</tr>
<tr>
<td>ssRNA</td>
<td></td>
</tr>
<tr>
<td>dsRNA</td>
<td></td>
</tr>
<tr>
<td>CpG</td>
<td></td>
</tr>
<tr>
<td>Proteins</td>
<td>Bacteria</td>
</tr>
<tr>
<td>Pilin</td>
<td></td>
</tr>
<tr>
<td>Flagellin</td>
<td></td>
</tr>
<tr>
<td>Cell wall lipids</td>
<td>Gram-negative bacteria, Gram-positive bacteria</td>
</tr>
<tr>
<td>LPS</td>
<td></td>
</tr>
<tr>
<td>Lipoteichoic acid</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Fungi, bacteria</td>
</tr>
<tr>
<td>Mannan</td>
<td></td>
</tr>
<tr>
<td>Dectin glucans</td>
<td></td>
</tr>
<tr>
<td>Damage-Associated Molecular Patterns</td>
<td></td>
</tr>
<tr>
<td>Stress-induced proteins</td>
<td>HSPs</td>
</tr>
<tr>
<td>Crystals</td>
<td>Monosodium urate</td>
</tr>
<tr>
<td>Nuclear proteins</td>
<td>HMGB1</td>
</tr>
</tbody>
</table>

CpG, cytidine-guanine dinucleotide; dsRNA, double-stranded RNA; HMGB1, high-mobility group box 1; HSPs, heat shock proteins; LPS, lipopolysaccharide; ssRNA, single-stranded RNA.

Different ligands bind to different receptors

PAMPs

Pattern recognition receptors (PRR)

Abbas et al, Cells and Molecular Immunology, 7 edition, 2012
Killing mechanisms of innate cells

Killing by phagocytosis

Killing by activation of macrophage from T cells
Active and passive immunity

**Vaccine**

- **Active immunity**
  - Microbial antigen (vaccine or infection)
  - Challenge infection
  - Recovery (immunity)
  - Specificity: Yes
  - Memory: Yes

- **Passive immunity**
  - Serum (antibodies) from immune individual
  - Infection
  - Recovery (immunity)
  - Specificity: Yes
  - Memory: No

*Abbas et al, Cells and Molecular Immunology, 7 edition, 2012*
Adaptive immunity

Need activation via at least 2 signals:

antigens + costimulators, complements or innate responses

Abbas et al, Cells and Molecular Immunology, 7 edition, 2012
T cell activation in adaptive immunity

2 signals
naive cells needs DC

Abbas et al, Cells and Molecular Immunology, 7 edition, 2012
CD28 and CD40L are essential for T cell activation

Abbas et al, Cells and Molecular Immunology, 7 edition, 2012
State and markers for T cell activation

A

![Graph showing the timeline of T cell activation with various markers and stages.]

B

- Retention in lymph node
- Proliferation
- Amplification and effector functions
- Control of response

- Naive T cell
- TCR
- CD69
- IL-2R (CD25)
- CD40L
- CTLA-4

Time after activation
Activation of T cells

T cell activation leads to differentiation of T cell subsets

Control of stimulation can be blocked by costimulatory blockade

Abbas et al, Cells and Molecular Immunology, 7 edition, 2012
Type of T cell responses

- **T\textsubscript{H}1** cell
  - Signature cytokines: IFN\textsubscript{\gamma}, IL-4, IL-5, IL-13
  - Immune reactions: Macrophage activation; IgG production
  - Host defense: Intracellular microbes
  - Role in diseases: Autoimmune diseases; tissue damage associated with chronic infections

- **T\textsubscript{H}2** cell
  - Signature cytokines: IL-4, IL-5, IL-13
  - Immune reactions: Mast cell, eosinophil activation; IgE production; "alternative" macrophage activation
  - Host defense: Helminthic parasites
  - Role in diseases: Allergic diseases

- **T\textsubscript{H}17** cell
  - Signature cytokines: IL-17A, IL-17F, IL-22
  - Immune reactions: Neutrophilic, monocytic inflammation
  - Host defense: Extracellular bacteria; fungi
  - Role in diseases: Organ-specific autoimmunity

*Abbas et al, Cells and Molecular Immunology, 7 edition, 2012*
Th1 help innate to clear intracellular pathogens
Th2 plays role in allergy and helminthic infections
Th17 cells are very important in inflammation and extracellular pathogens.
B cell activation

Recognition phase

Helper T cells, other stimuli

Activation phase: B cell proliferation and differentiation

Clonal expansion

Plasma cell

Antibody secretion

Isotype switching

Affinity maturation

Memory B cell

Resting IgM+ IgD+ mature B cell

Activated B cell

IgG-expressing B cell

High-affinity IgG

High-affinity IgG

TI, TD

No class switching (only IgM)

Abbas et al, Cells and Molecular Immunology, 7 edition, 2012
Activation of memory cells: important to generate vaccines
**T-dependent, isotype-switched, high-affinity antibodies; long-lived plasma cells**

- **Spleen, other lymphoid organs**
  - Follicular B cells
  - IgD + Protein antigen + helper T cell
  - Germinal center reaction
  - IgG, IgA, IgE

- **Marginal zone B cells**
  - IgM
  - Polysaccharides, lipids, etc.
  - T-independent, mainly IgM; short-lived plasma cells

- **Mucosal tissues, peritoneal cavity**
  - B-1 B cells
  - IgM
  - Polysaccharides, lipids, etc.
  - T-independent, mainly IgM; short-lived plasma cells

**T-independent, mainly IgM; short-lived plasma cells**

- Follicle
- B cell activation
- Long-lived plasma cells, memory B cells

- Effector T cells
- Dendritic cell
- T cell activation

- Initial T-B interaction
- Follicular dendritic cell
- Follicular helper T cell

- Short-lived plasma cells
- Extrafollicular focus
- Extrafollicular helper T cell

- Germinal center reaction
Vaccinology

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What is Vaccine?

- A vaccine is a non-pathogenic or attenuated antigen that mimics a particular pathogen in order to elicit an immune response.
- The goal of a vaccine is to establish immunity against that particular pathogen.
Principles of vaccination strategies

- **Purified antigens** → protective antibody
  - Not effective against microbes that mutate antigenic proteins or hide inside infected cells

- **Attenuated microbes, viral vectors for antigens** → antibodies + CMI
  - Safety concerns

- Difficult to induce effective CTL responses with purified protein antigens
  - Potential of plasmid DNA vaccines

- Clinically usable **adjuvants**
Vaccine: How it works

Antigens

APC: DC

T cells → B cells

Some cases, Ag stimulate directly to B cells
Approaches for vaccines
<table>
<thead>
<tr>
<th>Factor</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effectiveness</td>
<td>Must evoke protective levels of immunity:</td>
</tr>
<tr>
<td></td>
<td>at the appropriate site</td>
</tr>
<tr>
<td></td>
<td>of relevant nature (Ab, Tc, Th1, Th2)</td>
</tr>
<tr>
<td></td>
<td>of adequate duration</td>
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<tr>
<td>Availability</td>
<td>Readily cultured in bulk or accessible source of subunit</td>
</tr>
<tr>
<td>Stability</td>
<td>Stable under extreme climatic conditions, preferably not requiring refrigeration</td>
</tr>
<tr>
<td>Cheapness</td>
<td>What is cheap in the West may be expensive in developing countries but the Bill and Melinda Gates Foundation and governments help</td>
</tr>
<tr>
<td>Safety</td>
<td>Eliminate any pathogenicity</td>
</tr>
</tbody>
</table>
Vaccine Types

1. Killed whole organisms
In crude approach, the vaccine is made from the entire organism, killed to make it harmless. The typhoid vaccine is an example.

2. Attenuated organisms
Here, the organism has been cultured so as to reduce its pathogenicity, but still retain some of the antigens of the virulent form. The Bacillus Calmette-Guérin (BCG) is a weakened version of the bacterium that causes tuberculosis in cows. BCG is used as a vaccine against tuberculosis in many European countries but is rarely used in the U. S.

3. Toxoids
In some diseases, diphtheria and tetanus are notorious examples, it is not the growth of the bacterium that is dangerous, but the protein toxin that is liberated by it. Treating the toxin with, for example, formaldehyde, denatures the protein so that it is no longer dangerous, but retains some epitopes on the molecule that will elicit protective antibodies.

4. Surface molecules
Antibodies are most likely to be protective if they bind to the surface of the invading pathogen triggering its destruction. Several vaccines employ purified surface molecules.

5. Inactivated virus
Like killed bacterial vaccines, these vaccines contain whole virus particles that have been treated (again, often with formaldehyde) so that they cannot infect the host's cells but still retain some unaltered epitopes. The Salk vaccine for polio (IPV) is an example.
Vaccine Types

6. Attenuated virus

In these vaccines, the virus can still infect but has been so weakened that it is no longer dangerous. The measles, mumps, and rubella ("German measles") vaccines are examples. The Sabin oral polio vaccine (OPV) is another example.

7. DNA Vaccine

With DNA vaccines, the subject is not injected with the antigen but with DNA encoding the antigen. The DNA is incorporated in a plasmid containing DNA sequences encoding one or more protein antigens or, often, simply epitopes of the complete antigen(s); DNA sequences incorporating a promoter that will enable the DNA to be efficiently transcribed in the human cells. Sometimes DNA sequences encoding costimulatory molecules sequences that target the expressed protein to specific intracellular locations (e.g., endoplasmic reticulum) are included as well.

The DNA vaccine can be injected into a muscle just as conventional vaccines are.

In contrast to conventional vaccines, DNA vaccines elicit cell-mediated — as well as antibody-mediated — immune responses.
Type of vaccines

**Close to Nature. Virus:** easy,  
**Bacterial:** difficult

**Killed, Easy, safe and stable.**

**Only epitopes or antigens those recognized by T or B cells. Low side effects.**

**Inactivate toxins by treating them with formalin, a solution of formaldehyde and sterilized water.**

**Carbohydrate antigens with proteins**

**Naked DNA (or with particles) contained genes stimulate immune responses. (Herpes, West Nile, Influenza)**

**Use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. “Vector”**

From: Ilchmann et al, Harvard Sussex Program project examining the role of S&T reviews within the BWC with modifications.
Vaccine concepts

• Extracellular bacteria or toxin
  – Antibodies or B cells
  – Blocking antibodies
  – Complements
  – Need T cells for class switching.
  – Need conformational epitopes (B cells)

• Intracellular bacteria or virus
  – CMI or T cells
  – CTL
  – Activated macrophages
  – Cytokines
How to find the candidate antigens?

• Conventional approaches
  – Antigens selected from specific criteria (surface molecules and accessible).
  – Derived from basic investigations.

• Post genomic approaches
  – Reverse vaccinology
Antigen selections (1)

• Accessible to immune cells
  – Toxin, surface antigens

• Possess T and B cell epitopes

• Immunogenic in human (present or not present in nature)

• Important for survival and diseases causation of pathogens.
  – present in all/most of disease isolates.
  – loss/alteredation of disease survival
Antigen selections (2)

• Contain epitopes common to all/most isolates

• Target selection should based on
  – Pathogenesis of diseases
Efficacy of vaccines

• Vaccines have been useful for generating protective antibodies, but so far, not for generating effective cell-mediated immunity

• Vaccines work best against microbes that:
  - Do not vary their antigens
  - Do not have animal reservoirs
  - Do not establish latent infection within host cells
  - Do not interfere with the host immune response
Schematic view of conventional vaccinology and evolving vaccinology in the post-genome era.
Subunit vaccines

- Whole organisms have a multiplicity of antigens, some of which are not protective, may induce hypersensitivity or might even be immunosuppressive.
- It makes particular sense in these cases to use purified components or those made recombinantly.
- Toxoids, inactivated toxins, are effective as vaccines in preventing illness due to some bacterial agents.
- The hepatitis B surface antigen particle is a classic example of an effective subunit viral vaccine.
- Many successful bacterial vaccines target glycans on the surface of the organism using glycoconjugate preparations.
- DNA encoding the proteins from a pathogen can be injected directly into muscle injected directly into muscle to generate the proteins in situ and produce immune responses. The advantages are stability, ease of production and cheapness. The method has not been as effective in humans as in mice but newer developments such as a DNA prime with a protein or vector boost are promising.
Killed organisms as vaccines

- Killed bacteria and viruses have been widely used as effective vaccines.

Live attenuated organisms

- The advantages include the larger antigen dose typically provided by a replicating organism, the tendency to elicit better cellular immunity and the generation of an immune response at the site of the natural infection.
- Nonpathogenic vectors such as adenovirus, attenuated fowlpox and modified vaccinia Ankara virus can serve as Trojan horses for genes from pathogenic organisms that are difficult to attenuate.
- BCG is a good vehicle for antigens requiring CD4 T-cell immunity and salmonella constructs may give oral and systemic immunity. Intranasal immunization is gaining popularity.
- The risk with live attenuated organisms is reversion to the virulent form and danger to immunocompromised individuals.
Newer approaches to vaccines

- The rise of genomics has been crucial in allowing a rational approach to the identification of many more bacterial vaccine targets. "Reverse vaccinology" has been successfully applied to the development of a MenB vaccine.

- Highly variable pathogens such as HIV and HCV present particular problems to vaccine design in that they require the elicitation of broadly protective immune responses. Here molecular approaches are being adopted to describe how broadly neutralizing antibodies interact with their targets and use the information to rationally design vaccine candidates.
CONVENTIONAL VACCINOLOGY

Cultivate microorganism
antigen selection

- Antigen selection
- Identify components
- Purify components
- Test convalescent sera
- Test immunogenicity
- Clone genes
- Immunogenicity testing in animal models
- Vaccine development
- 5-15 years

THE GENOME-BASED APPROACH TO VACCINE DEVELOPMENT

- Genome sequence
- Proteomics technologies
- IVET, STM, DNA microarrays

- In silico analysis
- High throughput expression of recombinant proteins
- In vitro and in vivo assay for antigens identification
- Vaccine development
- VACCINE

MenB vaccine development

Preclinical reverse vaccinology

1998

2158 ORFs identified in the MenB MC58 genome

570 ORFs predicted to encode surface-expressed or secreted proteins

350 proteins expressed in E. coli, purified and used to immunize mice

91 novel surface-exposed proteins identified

28 novel proteins induced bactericidal antibodies

Serum bactericidal activity

% Survival after 60 min

Reciprocal of serum dilution

50% bactericidal killing

Bacterial cell no.

Fluorescence

5 proteins selected for use in a four-component vaccine formulation

fHBP-GNA2091

GNA2132-GNA1030

NadA

OMV
Neisseria meningitidis serogroup B

Reverse vaccinology

- Computer prediction of novel antigens (2158 ORFs)
- In silico vaccine candidates (600 ORFs)
- Express recombinant proteins (350 ORFs)
- Immunogenicity testing in animal models

Start from the whole genomic sequence
1-2 years

Vaccine development

Comparison of conventional and genomic approaches to vaccine development

<table>
<thead>
<tr>
<th>Conventional vaccinology</th>
<th>Reverse vaccinology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most abundant antigens during disease</td>
<td>All antigens immunogenic during disease</td>
</tr>
<tr>
<td>Antigens expressed in vitro</td>
<td>Antigens expressed in vitro and in vivo</td>
</tr>
<tr>
<td>Cultivable microorganism</td>
<td>Antigens even in non-cultivable microorganisms</td>
</tr>
<tr>
<td>Animal models essential</td>
<td>Animal models essential</td>
</tr>
<tr>
<td>Correlates of protection useful</td>
<td>Correlates of protection essential</td>
</tr>
<tr>
<td>Structural components of microorganism</td>
<td>Non-structural components, including early proteins of viruses</td>
</tr>
<tr>
<td>Polysaccharides may be used as antigens</td>
<td>Correct folding in recombinant expression important</td>
</tr>
<tr>
<td></td>
<td>High throughput expression/analysis important</td>
</tr>
<tr>
<td></td>
<td>Non-proteic antigens cannot be used</td>
</tr>
</tbody>
</table>

Problems with antigen selections

- No real comparison between different antigens.
- Limitation in ability to predict efficacy.
- Lack of adequate infection models.
- Function assay do not reflect in vivo conditions.
- Do not know the antigen variation or loss of antigens.
Technology for Vaccines

Next-generation technologies
- New adjuvants, structural vaccinology,
- synthetic biology, DNA and RNA

Reverse vaccinology
- C. difficile,
- E. coli,
- group A streptococcus,
- group B streptococcus,
- meningococcus serogroup B,
- S. aureus

Glycoconjugation
- Group B streptococcus,
- H. influenzae type B,
- meningococcus serogroups A, C, Y
- and W135,
- pneumococcus,
- S. aureus

Recombinant DNA
- Acellular pertussis,
- hepatitis B,
- human papilloma virus,
- Lyme disease

Empirical approach
- BCG,
- diphtheria,
- influenza,
- MMRV,
- pertussis,
- polio,
- rabies,
- smallpox,
- tetanus

Vaccines in the 21st century

increase life expectancy

Different age groups need different vaccinations

Some characteristics of an ideal vaccine

- Shows an impeccable safety profile in all populations, including young infants, the elderly and immunocompromised subjects (such as HIV–positive subjects)
- Elicits a high level of long-lived efficacy, including in young infants and the elderly
- Requires only a single dose (or at most two doses spaced fairly close together) to confer protection
- Stimulates protection within 2 weeks of administration
- Administrable without a needle and syringe; that is, orally, nasally or transcutaneously or with a needle-free injection device
- Administrable in combination with (in the same formulation) or concomitantly (coadministered) with other vaccines
- Can be manufactured in large scale and with quality control by relatively uncomplicated and economical processes
- Amenable to production in formulations that are resistant to high and low temperatures and therefore free from strict storage requirements

Myron M Levine & Marcelo B Sztein. Nature Immunology, 2004
Adjuvants

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What is an adjuvant?

- Adjuvants are the substances that essential for enhancing and directing the adaptive immune response to vaccine antigens.

- They enhance the either innate or adaptive immune responses.

- This response is mediated by two main types of lymphocytes, B and T cells.
<table>
<thead>
<tr>
<th>Year</th>
<th>Vaccine</th>
<th>Adjuvant and mechanism</th>
<th>Scientific findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1885</td>
<td>Rabies</td>
<td>ssRNA TLRs 7 and 8</td>
<td>Briegen describes endotoxin</td>
</tr>
<tr>
<td>1886</td>
<td></td>
<td></td>
<td>Coley shows tumor necrosis with bacterial extracts</td>
</tr>
<tr>
<td>1889</td>
<td>Typhoid</td>
<td>LPS, DNA TLRs 1, 2, 4, 5, 6 and 9</td>
<td></td>
</tr>
<tr>
<td>1911</td>
<td></td>
<td>Lipovaccine</td>
<td>More durable immune response to typhoid vaccine</td>
</tr>
<tr>
<td>1916</td>
<td>BCG for TB</td>
<td>DNA, lipoprotein TLRs 1, 2, 6 and 9</td>
<td></td>
</tr>
<tr>
<td>1921</td>
<td></td>
<td>Aluminum salts</td>
<td>Enhanced antibody responses to diphtheria vaccine</td>
</tr>
<tr>
<td>1926</td>
<td></td>
<td>Incomplete Freund’s adjuvant (IFA)</td>
<td>Enhanced cellular and antibody responses to TB</td>
</tr>
<tr>
<td>1937</td>
<td>Diphtheria, pertussis and tetanus</td>
<td>LPS, DNA TLRs 1, 2, 4, 5, 6 and 9</td>
<td></td>
</tr>
<tr>
<td>1942</td>
<td>Whole-cell influenza</td>
<td>ssRNA TLRs 7 and 8</td>
<td></td>
</tr>
<tr>
<td>1949</td>
<td>Inactivated polio vaccine</td>
<td>ssRNA TLRs 7 and 8</td>
<td></td>
</tr>
<tr>
<td>1955</td>
<td></td>
<td></td>
<td>LPS structure determined</td>
</tr>
<tr>
<td>1966</td>
<td></td>
<td></td>
<td>Ribi makes detoxified endotoxin MPL</td>
</tr>
<tr>
<td>1979</td>
<td></td>
<td></td>
<td>MPL tested in clinic</td>
</tr>
</tbody>
</table>

*Steven G Reed, Mark T Orr & Christopher B Fox Nature Medicine, 2013.*
<table>
<thead>
<tr>
<th>Year</th>
<th>Vaccine Type</th>
<th>Adjuvant Details</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>Fluad</td>
<td>MF59 (oil-in-water emulsion)</td>
<td>TLRs discovered</td>
</tr>
<tr>
<td>1997</td>
<td>Epaxal (for hepatitis A)</td>
<td>Virosome</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inflexal (for influenza)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td>LPS shown to be TLR ligand</td>
</tr>
<tr>
<td>2004</td>
<td>Invivac (for influenza; Europe)</td>
<td>Virosome</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>Fendrix (for hepatitis B; Europe)</td>
<td>MPL Defined TLR4</td>
<td></td>
</tr>
<tr>
<td>2007–2009</td>
<td>Pandemic influenza vaccines (Europe)</td>
<td>MF59, AS03 (oil-in-water emulsion)</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Cervarix (for HPV16 and HPV18; USA)</td>
<td>MPL Defined TLR4</td>
<td></td>
</tr>
</tbody>
</table>
Mechanism of Adjuvants

1. Adjuvants may exert their effects through different mechanisms.
2. Some adjuvants, such as alum and emulsions (e.g. MF59®), function as delivery systems.
3. Some providing slow release in order to continue the stimulation of the immune system.
4. Some enhance the antigen persistence at the injection site and increase recruitment and activation of antigen presenting cells (APCs). Some adjuvants are also capable of directing antigen presentation by the major histocompatibility complexes (MHC) [1].
5. Other adjuvants, essentially ligands for pattern recognition receptors (PRR), act by inducing the innate immunity, predominantly targeting the APCs and consequently influencing the adaptative immune response.
   - Toll-like receptors (TLRs),
   - NOD-like receptors (NLRs),
   - RIG-I-like receptors (RLRs) and
   - C-type lectin receptors (CLRs).
   - They signal through pathways that involve distinct adaptor molecules leading to the activation of different transcription factors. These transcription factors (NF-κB, IRF3)
   - Activation of some members of the NLR family, such as NLRP3 and NLRC4,
<table>
<thead>
<tr>
<th>Table I. Selective List of Different Classes of Adjuvants That Have Been Evaluated for Enhancing Immune Responses to Vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral salts</td>
</tr>
<tr>
<td>Aluminum phosphate*</td>
</tr>
<tr>
<td>Calcium phosphate*</td>
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<tr>
<td>Immunostimulatory adjuvants</td>
</tr>
<tr>
<td>Saponins e.g., QS21</td>
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<tr>
<td>MDP derivatives</td>
</tr>
<tr>
<td>Bacterial DNA (CpG oligos)</td>
</tr>
<tr>
<td>LPS</td>
</tr>
<tr>
<td>MPL and synthetic derivatives</td>
</tr>
<tr>
<td>Lipopeptides</td>
</tr>
<tr>
<td>Lipid particles</td>
</tr>
<tr>
<td>Liposomes</td>
</tr>
<tr>
<td>Virosomes*</td>
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<tr>
<td>Iscoms</td>
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<tr>
<td>Cochleates</td>
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<tr>
<td>Particulate adjuvants</td>
</tr>
<tr>
<td>Poloxamer particles</td>
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<tr>
<td>Virus-like particles</td>
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<tr>
<td>Mucosal adjuvants</td>
</tr>
<tr>
<td>Cholera toxin (CT)</td>
</tr>
<tr>
<td>Mutant toxins e.g., LTK63 and LTR72</td>
</tr>
<tr>
<td>Microparticles</td>
</tr>
<tr>
<td>Polymerized liposomes</td>
</tr>
<tr>
<td>Chitosan</td>
</tr>
<tr>
<td>Aluminum hydroxide*</td>
</tr>
<tr>
<td>Cytokines e.g., IL-2, IL-12, GM-CSF</td>
</tr>
<tr>
<td>Emulsions e.g., Freund’s, SAF, MF59*</td>
</tr>
<tr>
<td>PLG microparticles</td>
</tr>
<tr>
<td>Heat labile enterotoxin (LT)</td>
</tr>
</tbody>
</table>

Manmohan Singh and Derek T. O'Hagan, 2002
Mechanisms of Adjuvants
## Adjuvants

<table>
<thead>
<tr>
<th>Adjuvant name (year licensed)</th>
<th>Adjuvant class</th>
<th>Components</th>
<th>Vaccines (disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adjuvants licensed for use in human vaccines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alum* (1924)</td>
<td>Mineral salts</td>
<td>Aluminium phosphate or aluminium hydroxide</td>
<td>Various</td>
</tr>
<tr>
<td>MF59 (Novartis; 1997)</td>
<td>Oil-in-water emulsion</td>
<td>Squalene, polysorbate 80 (Tween 80; ICI Americas), sorbitan trioleate (Span 85; Croda International)</td>
<td>Fluad (seasonal influenza), Focetria (pandemic influenza), Aflunov (pre-pandemic influenza)</td>
</tr>
<tr>
<td>AS03 (GlaxoSmithKline; 2009)</td>
<td>Oil-in-water emulsion</td>
<td>Squalene, Tween 80, α-tocopherol</td>
<td>Pandremix (pandemic influenza), Prepandrix (pre-pandemic influenza)</td>
</tr>
<tr>
<td>Virosomes (Berna Biotech; 2000)</td>
<td>Liposomes</td>
<td>Lipids, hemagglutinin</td>
<td>Inflexal (seasonal influenza), Epaxal (hepatitis A)</td>
</tr>
<tr>
<td>AS04* (GlaxoSmithKline; 2005)</td>
<td>Alum-absorbed TLR4 agonist</td>
<td>Aluminium hydroxide, MPL</td>
<td>Fendrix (hepatitis B), Cervarix (human papilloma virus)</td>
</tr>
<tr>
<td><strong>Vaccine adjuvants tested in humans but not licensed for use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CpG 7909, CpG 1018</td>
<td>TLR9 agonist</td>
<td>CpG oligonucleotides alone or combined with alum/emulsions</td>
<td>−</td>
</tr>
<tr>
<td>Imidazoquinolines</td>
<td>TLR7 and TLR8 agonists</td>
<td>Small molecules</td>
<td>−</td>
</tr>
<tr>
<td>Polyl:C</td>
<td>TLR3 agonist</td>
<td>Double-stranded RNA analogues</td>
<td>−</td>
</tr>
<tr>
<td>Pam3Cys</td>
<td>TLR2 agonist</td>
<td>Lipopeptide</td>
<td>−</td>
</tr>
<tr>
<td>Flagellin</td>
<td>TLR5 agonist</td>
<td>Bacterial protein linked to antigen</td>
<td>−</td>
</tr>
<tr>
<td>Iscomatrix</td>
<td>Combination</td>
<td>Saponin, cholesterol, dipalmitoylphosphatidylcholine</td>
<td>−</td>
</tr>
<tr>
<td>AS01</td>
<td>Combination</td>
<td>Liposome, MPL, saponin (QS21)</td>
<td>−</td>
</tr>
<tr>
<td>AS02</td>
<td>Combination</td>
<td>Oil-in-water emulsion, MPL, saponin (QS21)</td>
<td>−</td>
</tr>
<tr>
<td>AF03</td>
<td>Oil-in-water emulsion</td>
<td>Squalene, Montane 80, Eumulgin B1 PH</td>
<td>−</td>
</tr>
<tr>
<td>CAF01</td>
<td>Combination</td>
<td>Liposome, DDA, TDB</td>
<td>−</td>
</tr>
<tr>
<td>IC31</td>
<td>Combination</td>
<td>Oligonucleotide, cationic peptides</td>
<td>−</td>
</tr>
<tr>
<td>Adjuvants</td>
<td>Formulation</td>
<td>In pre-clinical or clinical trials</td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Montanides</td>
<td>Water-in-oil emulsions</td>
<td>Malaria (Phase I), HIV, cancer (Phase I/II)</td>
<td></td>
</tr>
<tr>
<td>Saponins (QS-21)</td>
<td>Aqueous</td>
<td>Cancer (Phase II), herpes (Phase I), HIV (Phase I)</td>
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</tr>
<tr>
<td>SAF</td>
<td>Oil-in-water emulsion containing squalene, Tween™ 80, Pluronic™ L121</td>
<td>HIV (Phase I – Chiron)</td>
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</tr>
<tr>
<td>AS03</td>
<td>Oil-in-water emulsion containing α-tocopherol, squalene, Tween™ 80</td>
<td>Pandemic flu (GSK)</td>
<td></td>
</tr>
<tr>
<td>MTP-PtdEtn</td>
<td>Oil-in-water emulsion</td>
<td>HSV</td>
<td></td>
</tr>
<tr>
<td>Exotoxins</td>
<td>P. aeruginosa</td>
<td>P. aeruginosa, cystic fibrosis (AERUGEN – Crucell/Berna)</td>
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</tr>
<tr>
<td></td>
<td>E. coli heat-labile enterotoxin LT</td>
<td>ETEC (Phase II – Iornai Corp.)</td>
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</tr>
<tr>
<td>ISCOMS</td>
<td>Phospholipids, cholesterol, QS-21</td>
<td>Influenza, HSV, HIV, HBV, malaria, cancer</td>
<td></td>
</tr>
<tr>
<td>TLR ligands</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MPL®-SE</td>
<td>Oil-in-water emulsion</td>
<td>Leishmania (Phase I/II – IDRI)</td>
<td></td>
</tr>
<tr>
<td>Synthetic Lipid A</td>
<td>Oil-in-water emulsion</td>
<td>Various indications (Avanti/IDRI)</td>
<td></td>
</tr>
<tr>
<td>MPL®-AF</td>
<td>Aqueous</td>
<td>Allergy (ATL); cancer (Biomira)</td>
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</tr>
<tr>
<td>AS01</td>
<td>Liposomal</td>
<td>HIV (Phase I), malaria (AS01, Phase III, GSK) cancer (Phase II/III, Biomira/MerckKGaA)</td>
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<tr>
<td>AS02</td>
<td>Oil-in-water emulsion containing MPL® and QS-21</td>
<td>HPV (Cervarix), HIV, tuberculosis, malaria (Phase III), herpes (GSK)</td>
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</tr>
<tr>
<td>AS04</td>
<td>Alum + aqueous MPL®</td>
<td>HPV, HAV (GSK)</td>
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</tr>
<tr>
<td>AS15</td>
<td>AS01 + CpG</td>
<td>Cancer therapy (GSK)</td>
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<tr>
<td>RCS29</td>
<td>Aqueous</td>
<td>HBV, pneumovax</td>
<td></td>
</tr>
<tr>
<td>TLR-9</td>
<td>n/a</td>
<td>Cancer (ProMune – Coley/Pfizer)</td>
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<tr>
<td>(CpG)</td>
<td></td>
<td>HCV (ACTILON Coley)</td>
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<tr>
<td>TLR-9 ISS series</td>
<td>n/a</td>
<td>HIV, HBV, HSV, anthrax (VaxImmune Coley/GSK/Chiron)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HBV (HEPLISAV, Phase III – Dynavax)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Cancer (Phase II, Dynavax)</td>
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<tr>
<td>TLR-9 IMO series</td>
<td>n/a</td>
<td>Cancer (IMOxine, Phase I, Hybridon Inc.)</td>
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<tr>
<td>(YpG, CpR motif)</td>
<td>n/a</td>
<td>Cancer (IMO-2055, Phase II, Idera Pharm.)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>HIV (Remune, Phase I, Idera/IMNR)</td>
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</tr>
<tr>
<td>TLR-9 agonist (MIDGE®)</td>
<td>n/a</td>
<td>Cancer (Phase I, Mologen AG)</td>
<td></td>
</tr>
<tr>
<td>TLR-7/8 (Imiquimod)</td>
<td>n/a</td>
<td>Melanoma (3M Pharmaceutical)</td>
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</tr>
</tbody>
</table>

Reed, S.G et al, 2008
Adjuvants: as innate stimulators

Alan R. Shaw, Mark B. Feinberg, 2009
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