Ch 18
Practical Applications of Immunology
LEARNING OBJECTIVES

Define vaccine and explain why vaccination works

Differentiate between attenuated, inactivated, toxoid, subunit, and conjugated vaccines. Provide an example of each

Compare and contrast the production of whole-agent vaccines, recombinant vaccines, and DNA vaccines.

Define adjuvant.

Explain the value of vaccines, and discuss acceptable risks for vaccines.

Explain how antibodies are used to diagnose diseases.

Define monoclonal antibodies, explain how they are made, and identify their advantage over polyclonal antibodies.

Differentiate between precipitation and direct and indirect agglutination.

Explain neutralization and the hemagglutination inhibition test.

Compare and contrast direct and indirect fluorescent-antibody tests.

Explain how direct and indirect ELISA tests work.
Vaccine History: from

- **Variolation**: Inoculation smallpox into skin (18th century).

  
  to

- **Vaccination**: Edward Jenner developed the modern practice of vaccination when he inoculated people with cowpox virus to protect them against smallpox.
Herd Immunity

Large proportion of group is resistant
⇒ whole group is resistant

- Results from effective vaccination programs
  (90% vaccinated → no spread of disease)

- Factors affecting herd immunity
  - Environment (rural vs. city life)
  - Individual’s immune system

- Herd immunity animation
Types of Vaccines and Their Characteristics

**Attenuated whole-agent** vaccine: attenuated (weakened) microorganisms (or virus) → generally provides lifelong immunity.

**Inactivated whole-agent** vaccine: killed bacteria or viruses

**Toxoid** vaccine (inactivated toxin)

**Subunit vaccine**: antigenic fragments of a microorganism; includes recombinant and acellular vaccines.

**Conjugated vaccine** combines the desired antigen with a protein that boosts the immune response.

**DNA** (= genetic immunization) stimulate humoral and cellular immunity; thermostable; easy to produce. In clinical trials.

**Booster Immunizations**

**Adjuvants** improve effectiveness of some antigens (increase availability of ag in lymph system)
Vaccination Strategy: Polio

Paralytic poliomyelitis (caused by one of three serotypes of the polio virus)

- Mid 1950s – Salk vaccine (inactivated virus)
- 1962 – Sabin vaccine (attenuated virus)
- Salk vaccine is safer but no mucosal immunity
- Sabin vaccine provides herd immunity
Principal Vaccines Used in the US to Prevent Bacterial Diseases in Humans

- **DtaP**
  - Diphtheria: Purified diphtheria toxoid
  - Pertussis: Acellular fragments of *B. pertussis*
  - Tetanus: Purified tetanus toxoid

- **Meningococcal meningitis**: Purified polysaccharide from *N. meningitidis*

- **Haemophilus influenzae** type b meningitis: Polysaccharides conjugated with protein

- Pneumococcal conjugate vaccine: *S. pneumoniae* antigens conjugated with protein
Principal Vaccines Used in the US to Prevent Viral Diseases in Humans

- **Smallpox**: Live vaccinia virus
- **Poliomyelitis**: Inactivated virus
- **Rabies**: Inactivated virus
- **Hepatitis A**: Inactivated virus
- **Influenza**: Inactivated or attenuated virus
- **Measles**: Attenuated virus
- **Mumps**: Attenuated virus
- **Rubella**: Attenuated virus
- **Chickenpox**: Attenuated virus
- **Hepatitis B**: Antigenic fragments (recombinant vaccine)
### Recommended Immunization Schedule for Persons Aged 0 Through 6 Years—United States • 2010

For those who fall behind or start late, see the catch-up schedule.

<table>
<thead>
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<th>Vaccine ▼</th>
<th>Age ▼</th>
<th>Birth</th>
<th>1 month</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
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This schedule includes recommendations in effect as of December 15, 2009. Any dose not administered at the recommended age should be administered at a subsequent visit, when indicated and feasible. The use of a combination vaccine generally is preferred over separate injections of its equivalent component vaccines. Considerations should include provider assessment, patient preference, and the potential for adverse events. Providers should consult the relevant Advisory Committee on Immunization Practices statement for detailed recommendations: [http://www.cdc.gov/vaccines/pubs/acip-list.htm](http://www.cdc.gov/vaccines/pubs/acip-list.htm). Clinically significant adverse events that follow immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS) at [http://www.vaers.hhs.gov](http://www.vaers.hhs.gov) or by telephone, 800-822-7967.

Compare to Table 18.3
Tests based on the interactions of antibodies and antigens

These tests determine the presence of antibodies or antigens in a patient.

**Sensitivity**: Determined by the percentage of positive samples it correctly detects

**Specificity**: Determined by the percentage of false positive results it gives.
Monoclonal Antibodies (MAbs)

- Pathogens contain many different antigens (and many more epitopes) ⇒ **Polyclonal antibody** response

- **Monoclonal antibody**: single type of antibody directed against one specific epitope. Produced by single B cell clone.

- **Generation of MAbs**: Hybridoma production in the laboratory by fusing a cancerous cell with an antibody-secreting plasma cell.

- The hybridoma cell culture produces large quantities of the plasma cell’s antibodies (monoclonal antibodies).
MAbs are used:

- for serological identification (tissue and blood typing)
- to prevent tissue rejection
- as immunotoxins to treat cancer
- to measure serum protein and drug levels
- to identify infectious agents
- to identify and quantify hormones

Compare to Foundation Fig 18.2
Development of New Generations of MAbs

- Chimeric MAbs (66% human): Genetically modified mice produce Ab with a human constant region.
- Humanized mabs (90% human): MAbs that are mostly human, except for mouse antigen-binding.
- Fully human antibodies: MAbs produced from mice with human antigen genes.
- Bacterial, plant and animal systems under investigation to increase production volumes.
Precipitation Reactions

Interaction of **soluble antigens** with IgG or IgM antibodies.

When optimal proportions of antigens and antibodies \(\rightarrow\) **Lattice formation**. Excess of either component decreases lattice formation and subsequent precipitation.

Precipitin ring test performed in small tube. **Immunodiffusion** procedures: precipitation reactions carried out in agar gel medium.
Agglutination Reactions

- Interaction of particulate antigens and antibodies.

- Antigens may be
  - On a bacterial cell: **direct agglutination**.
  - Attached to latex spheres: **indirect** or passive **agglutination**.  
    *E.g.*: *syphilis test*

- Diseases may be diagnosed by combining the patient’s serum with a known antigen.
**Agglutination Reactions cont.**

- **Titer**: Concentration of antibodies against a particular antigen.

- **Seroconversion**: Rising antibody titer (from no antibodies to the presence of antibodies). Antibody levels are now detectable.

- Agglutination reactions are used in blood typing, the diagnosis of certain diseases, and the identification of viruses.
Indirect Agglutination Tests

*e.g.*: Syphilis Antibody Test

Syphilis Polymer Particles + Patient Serum with Anti-*T*.p allidum Antibodies = ID-PaGIA Agglutination
Neutralization Reactions

- Harmful effect of a bacterial exotoxin or virus is eliminated by a specific antibody (antitoxins and virus neutralization).
- Some viruses (mumps, measles, influenza) agglutinate RBCs in vitro.
- Antibodies against these viruses can be detected by their ability to interfere with viral hemagglutination.

Fig 18.8

Red blood cells + Viruses → Hemagglutination
Viral Hemagglutination-Inhibition Tests

- Hemagglutination involves agglutination of RBCs
- Some viruses agglutinate RBCs in vitro
- Antibodies prevent hemagglutination

![Diagram showing the process of hemagglutination inhibition](Fig 18.9b)
Fluorescent Antibody Techniques

- Use antibodies labeled with fluorescent dyes.
- **Direct fluorescent-antibody tests:** identify specific microorganisms.
- **Indirect fluorescent-antibody (IFA) tests:** demonstrate the presence of antibodies in serum.
1 Antibody is adsorbed to well.

2 Patient sample is added; complementary antigen binds to antibody.

3 Enzyme-linked antibody specific for test antigen is added and binds to antigen, forming sandwich.

4 Enzyme's substrate is added, and reaction produces a product that causes a visible color change.

For more details of ELISA testing see lab