History

• **Variolation** was the first method to be used to immunize people against smallpox (Variola virus)
  - Exposure to live virus

• **Vaccination** was introduced by Edward Jenner (1798)
  - Stemmed from the observation that those that contracted cowpox did not get smallpox later
Types of vaccines

- **Live attenuated vaccine**
  - Lifelong immunity is usually achieved without a booster vaccination
  - Activates both cell- and antibody-mediated immunity

- **Inactivated killed vaccine**
  - Usually killed by formalin or phenol
  - Vaccines against rabies, polio, influenza, cholera, pneumococcal pneumonia
  - Usually require booster doses
  - Induce mostly antibody-mediated immunity

**Subunit vaccines**

- Use antigenic fragments that best stimulate an immune response

- **Recombinant vaccines**
  - Other microbes are programmed to produce the antigenic fragments
  - i.e. Hepatitis B

- **Virus-like particle (VLP) vaccines**
  - Resemble viruses but no viral genetic material
  - i.e. HPV

**Subunit vaccines**

- **Toxoids (inactivated toxins)**
  - Vaccines are directed at the toxins produced by a pathogen
  - Requires a series of injections to achieve full immunity and later boosters
  - i.e. tetanus, diphtheria

- **Antitoxins**
  - Serum containing antibodies against toxin
Conjugated vaccines

- Attach a poor (i.e. polysaccharide) antigen to a carrier protein (i.e. diphtheria or tetanus toxoid), thereby conferring the immunological attributes of the carrier to the antigen
- Primarily used for the prevention of bacterial infections
- Vaccine for *Haemophilus influenzae* type B

Nucleic Acid (DNA) Vaccines

- Plasmid DNA is injected into host cells and then incorporated into the host DNA
- Antigens are then produced which can then stimulate both humoral and cellular immunity
- i.e. vaccine against equine West Nile virus

Diagnostic Immunology

- Sensitivity and specificity are features of modern diagnostic tests
- **Sensitivity** is the probability that the test is reactive if the subject is a true positive
- **Specificity** is the probability that the test will not give false positive results

Tuberculin skin test
**Diagnostic Immunology**

- Known antibodies can be used to identify unknown pathogens
- Known pathogens can be used to identify unknown antibodies
- Let's look at exams of antibody-based diagnostic tests

**Hybridoma**

- Myelomas are cancerous B cells that can be maintained in culture
- A hybridoma is formed when an antibody – producing B cell is fused with cells derived from a myeloma

**Formation of monoclonal antibodies**

**Monoclonal antibodies (Mabs)**

- Have a variety of therapeutic actions
- In certain inflammatory disorders, TNF is released. Mabs can neutralize the TNF and block progression of the disease.
- Mabs can block receptor sites
- Problem: some patients react to these “mouse” proteins
Monoclonal antibodies (Mabs)

- Chimeric Mabs
  - Humanized antibodies – minimize the murine component of chimeric Mab
  - Fully human antibodies – produced by mice which contain human antibody genes
- Spelling is an indicator of the origin of the Mab
  - Human (u), mouse (o), chimera (xi), humanized (zu)
  - -zumab (humanized), -omab (mouse), -ximab (chimera)
- Spelling can also be an indicator of what Mab is treating

Precipitation reactions

- Involve the reaction of soluble antigens with IgG or IgM antibodies to form “lattices”
- In the first stage, there is formation of small antigen-antibody complexes
- Over minutes to hours lattices will precipitate out of solution, but only if the ratio of antigen to antibody is optimal
**Immunodiffusion tests**
- Precipitation reactions carried out in an agar gel medium
- i.e. Ouchterlony double immunodiffusion assay
- Immunoelectrophoresis

**Agglutination reactions**
- Involve the use of particulate antigens or soluble antigens adhering to particles
- Antigen – antibody interactions form visible aggregates (agglutination)

**Agglutination reaction**

**Direct agglutination tests**
- Detect antibodies against relatively large cellular antigens, such as those on RBCs, bacteria, and fungi
**Indirect agglutination tests**

(a) Reaction in a positive indirect test for antibodies. When particles (latex beads here) are coated with antigens, agglutination indicates the presence of antibodies, such as the IgM shown here.

(b) Reaction in a positive indirect test for antigens. When particles are coated with monoclonal antibodies, agglutination indicates the presence of antigen.

**Viral hemagglutination**

Red blood cells + Viruses → Hemagglutination

**Neutralization**

(a) The effects of a toxin on a susceptible cell and neutralization of the toxin by antitoxin

Toxin molecules + Cell → Cell damaged by toxin

Toxin molecules + Antibodies to toxin (antitoxin) + Cell → Neutralized toxin and undamaged cell
In vitro neutralization tests

- Test serum is added to virus-infected cells in cell culture or embryonated eggs
- If the serum contains antibodies to the virus, it will prevent the virus from infecting cells in culture or eggs
- Can be used to identify virus and viral antibody titer

Viral hemagglutination inhibition test

Complement fixation

- Indicates the presence of antibodies to a known antigen
- Can detect very small amounts of antibody

Fluorescent-Antibody (FA) techniques

- Can identify microbes in clinical specimens
- Can detect the presence of a specific antibody in serum
- Fluorescent dyes (i.e. fluorescein isothiocyanate – FITC) combined with antibodies, allowing them to fluoresce with UV light
Direct FA tests

Indirect FA tests

Fluorescence-activated cell sorter (FACS)
• Applications include sexing of sperm

Enzyme – Linked Immunosorbant Assay (ELISA)
• Direct ELISA detects antigens
• Indirect ELISA detects antibodies
ELISA and home pregnancy tests

Fig. 18.13

Direct ELISA

Fig. 18.14a

Indirect ELISA

Fig. 18.14b

Western blots