Immunization and Testing

Topics
- Allergies
- Autoimmunity
- Immunodeficiency

Specific Immunities

- Active
- Passive
- Natural
- Artificial
- Vaccines

Active
- Natural or artificial
- Antigen activates B and T cells
- Memory cells
- Long-term protection
Passive

- Natural or artificial
- Receive antibodies from another individual or animal
- No memory cells
- No antibody production
- Short-term protection

Natural

- Immunity produced by normal biological experiences, no medical intervention
  - Natural active
    - Infection
  - Natural passive
    - Mother to child

Artificial

- Immune protection through medical procedures or intervention
  - Artificial active
    - Vaccination
  - Artificial passive
    - Immunotherapy
Summary: Acquired immunities

Immunization

1796 – Edward Jenner discovered process of vaccination
1879 – Louis Pasteur developed a vaccine against Pasteurella multocida

Antibody transfer developed when it was discovered vaccines protected through the action of antibodies

Immunization Effects
Vaccines

- Types
  - Killed whole cell or inactivated viruses
  - Live, attenuated cells or viruses
  - Antigenic molecules from bacteria or viruses
  - Genetically engineered microbes or microbial antigens

Attenuated (Live) Vaccine

- Use pathogens with reduced virulence
- Can result in mild infections
- Active microbes stimulate a strong immune response
- Can provide contact immunity
- Modified microbes can retain enough residual virulence to cause disease

Inactivated (Killed) Vaccine

- Whole agent vaccines
- Subunit vaccines
  - Both safer than live vaccines
- Antigenically weak because microbes don’t provide many antigenic molecules to stimulate the immune response
- Often contain adjuvants (increase effective antigenicity)
Toxoid Vaccine

- Chemically or thermally modified toxins used to stimulate active immunity
- Useful for some bacterial diseases
- Stimulate antibody-mediated immunity
- Require multiple doses because they possess few antigenic determinants

New vaccines

- Combinations
- Recombinant Gene Technology
  - Example: DNA vaccines
    - Insert microbial DNA into plasmid
    - Inoculate recipient with plasmid
    - Host cell expresses microbial DNA
    - Immune system reacts to microbial antigen expressed on the host cell surface

The steps associated with the preparation of DNA vaccines.
Benefits of vaccinations

- Long-lasting immunity
- Herd immunity
  - Indirect protection for non-immune
  - Prevents epidemics

In the U.S., there is a recommended childhood and adolescent immunization schedule.

Active Immunization & Safety

Problems associated with immunization:
- Mild toxicity most common
- Risk of anaphylactic shock
- Residual virulence from attenuated viruses
- Allegations certain vaccines cause autism, diabetes, and asthma
  - Research has not substantiated these allegations
Passive Immunotherapy

- Administration of antiserum containing preformed antibodies
- Immediate protection
- Antiserum limitations:
  - Contain antibodies against many antigens
  - Can trigger allergic reactions called serum sickness
  - Viral pathogens can contaminate antisera
  - Antibodies of antisera are degraded relatively quickly
- Use Hybridomas to overcome limitations...

About Hybridomas

- Mice are infected with antigen.
- Plasma cells, which secrete antibodies, are removed.
- Antibodies are harvested.
- Hybridomas are formed by mixing myeloma cells and lymphocytes from spleen cells of infected mice.
- Hybridomas are cultured in standard culture media and then antibodies are secreted by hybridoma cells.
- A hybridoma that makes antibodies that react with the antigen is cloned.

Comparing Passive Immunotherapy w/ Active Immunization

- Passive immunotherapy: administered at injection, has steady antibody levels, requires boosters
- Active immunization: initial inoculation, antibody levels increase over time, requires boosters
Immune Testing

- Uses serology
-- Study and diagnostic use of antigen-antibody interactions in blood serum
- Two categories of immune testing:
-- **Direct** testing (looks for antigens)
-- **Indirect** testing (looks for antibodies)
- How to choose? Consider suspected diagnosis, cost, and speed of result

Precipitation Tests

- Easy serological tests
- Antigens and antibody mixed in the proper proportion form large complexes called precipitates
- Immunodiffusion
  - Determines optimal antibody and antigen concentrations

Precipitation Reactions

![Precipitation Reactions](image)
Immunodiffusion (uses precipitation)

Determining Concentration

Radial Immunodiffusion

More Precipitation Testing

- Radial immunodiffusion
  - measure specific antibodies in serum
  - Produces anti-antibodies
  - The human antibodies are the “antigen” in the test, and the antibody is anti-human antibody
Agglutination Tests

- Agglutination → cross-linking of antibodies with particulate antigens
  - Agglutination = clumping of insoluble particles
  - Precipitation = aggregation of soluble molecules
- Reactions are easy to see and interpret with the unaided eye
- Hemagglutination
  - Agglutination of red blood cells
  - Can be used to determine blood type

Hemagglutination in Blood Typing

Use agglutination to quantify antibody
Neutralization Tests

- Viral neutralization
  - Cytopathic effect
    - Viruses introduced into appropriate cell cultures will kill the cells
  - Ability of virus to kill culture cells is neutralized when virus is first mixed with antibodies against it
  - Absence of cytopathic effect indicates presence of antibodies
  - Test can identify whether individual has been exposed to a particular virus or viral strain

Neutralization Tests

Example = Viral hemagglutination inhibition test
- Useful for viruses that aren’t cytopathic
- Based on viral hemagglutination
  - Ability of viral surface proteins to clump red blood cells
- Individual’s serum will stop viral hemagglutination if the serum contains antibodies against the specific virus
- Commonly used to detect antibodies against influenza, measles, and mumps

The Complement Fixation Test

- Based on generation of membrane attack complexes during complement activation
- Used to detect the presence of specific antibodies in an individual’s serum
- Can detect antibody amounts too small to detect by agglutination
Labeled Antibody Test

- Uses antibody molecules linked to some “label” (easily detected)
- Used to detect either antigens or antibodies

Example = Fluorescent antibody tests

- Use fluorescent dyes as labels
- Fluorescein is one dye used in these tests
- Fluorescein-labeled antibodies used in two types of tests
  - Direct fluorescent antibody tests
  - Indirect fluorescent antibody tests
Labeled Antibody Test

ELISA
- ELISA = Enzyme-Linked Immuno-Sorbent Assay
- Uses an enzyme as the label
  - Reaction of enzyme with its substrate produces a colored product indicating a positive test
- ELISA is commonly used to detect the presence of antibodies in serum

The ELISA

Labeled Antibody Test
- Antibody sandwich ELISA
  - Modification of the ELISA technique
  - Commonly used to detect antigen
  - Antigen being tested for is “sandwiched” between two antibody molecules
An antibody sandwich ELISA

- Can detect either antibody or antigen
- Can quantify amounts of antigen or antibody
- Easy to perform and can test many samples quickly
- Plates coated with antigen and gelatin can be stored for later testing

Labeled Antibody Test - ELISA

- Western blot test
  - Technique to detect antibodies against multiple antigens
  - Can detect more types of antibodies and is less subject to misinterpretation than other tests

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Recent Developments in Antibody-Antigen Immune Testing

- Simple immunoassays, results in minutes
- Useful in determining a preliminary diagnosis
- Immunofiltration and immunochromatography are most common
- Immunofiltration
  - Rapid ELISA that uses antibodies bound to membrane filters instead of polystyrene plates
  - Membrane filters have large surface area, making the assay quicker to complete

Recent Developments in Immune Testing

Immunochromatography

- Very rapid and easy to read ELISAs
- Antigen solution flows through a porous strip, where it encounters labeled antibody
- Visible line produced when antigen-antibody immune complexes encounter antibody against them
- Used in pregnancy testing to detect human chorionic growth hormone
Immunochromatographic dipstick

- Immunoassay strip
- Antigen-antibody complex
- Positive control
- Negative control

Figure 17.16

Prepared antigens extracted from patient's sample

Line of test
Line of control
对照 line

Movement of fluid containing complexes of antibodies and antigen