Chapter 18: Applications of Immunology

1. Vaccinations
2. Monoclonal vs Polyclonal Ab
3. Diagnostic Immunology
1. Vaccinations
What is Vaccination?

A method of inducing artificial immunity by exposing the individual to some portion or form of the pathogen (aka “immunization”):

- triggers an adaptive immune response resulting in the production of memory T and B cells specific for antigens from the pathogen

- a secondary exposure will result in a potent and immediate immune response to the specific pathogen due to the memory cells

**the vaccination itself should NOT cause an infection**
Different Types of Vaccines

1) **Attenuated** whole agent vaccines:
   - live but “weakened” pathogen
     - genetically modified
     - mutant or less virulent strains of the pathogen

2) **Inactivated** whole agent vaccines:
   - pathogen that has been killed in some way
     - usually by chemical treatment, also by heat

**whole agents are generally more effective due to containing multiple antigens, but also carry more risk of infection**
3) **Toxoid vaccines:**
   - chemically inactivated protein exotoxins
   - inactivated toxins are referred to as toxoids

4) **Subunit vaccines:**
   - a specific protein or protein fragment from pathogen
     - purified from pathogen directly OR
     - produced as a recombinant vaccine in other organism

5) **Conjugated vaccines:**
   - small or non-protein antigens attached to a “carrier”
     - necessary to enhance immune response

**these “molecular vaccines” tend to be less effective however they are safer than whole agents**
Haptens & Conjugated Antigens

- Some molecules (haptens) are too small to induce an IR
  - cannot be processed and presented on MHC class II
- Others (e.g. polysaccharides) are not very immunogenic
- In either case, conjugation to a protein carrier is nec.
Methods of Vaccine Production

1) growth & purification of pathogen itself
   • e.g., culturing bacteria, growing viruses in eggs
     • treated and packaged after purification

2) production of recombinant antigen
   • typically in yeast or bacteria
     • gene encoding protein antigen placed in plasmid
     • expressed in bacterial or yeast host cells
     • protein is purified & used in vaccine
Some Bacterial Vaccines

<table>
<thead>
<tr>
<th>Disease</th>
<th>Vaccine</th>
<th>Recommendation</th>
<th>Booster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria</td>
<td>Purified diphtheria toxoid</td>
<td>See Table 18.3</td>
<td>Every 10 years for adults</td>
</tr>
<tr>
<td>Meningococcal meningitis</td>
<td>Purified polysaccharide from <em>Neisseria meningitidis</em></td>
<td>For people with substantial risk of infection. Recommended for college freshmen, especially if living in dormitories.</td>
<td>Need not established</td>
</tr>
<tr>
<td>Pertussis (whooping cough)</td>
<td>Killed whole oracellular fragments of <em>Bordetella pertussis</em></td>
<td>Children prior to school age; see Table 18.3</td>
<td>For high-risk adults Available for ages 10–18 years</td>
</tr>
<tr>
<td>Pneumococcal pneumonia</td>
<td>Purified polysaccharide from 7 strains of <em>Streptococcus pneumoniae</em></td>
<td>For adults with certain chronic diseases; people over 65; children 2–23 months</td>
<td></td>
</tr>
<tr>
<td>Tetanus</td>
<td>Purified tetanus toxoid</td>
<td>See Table 18.3</td>
<td>Every 10 years for adults</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em> type b meningitis</td>
<td>Polysaccharide from *Haemophilus influenzae type b conjugated with protein to enhance effectiveness</td>
<td>Children prior to school age; see Table 18.3</td>
<td>None recommended</td>
</tr>
</tbody>
</table>

- bacterial vaccines are generally not as effective as viral vaccines
- bacterial antigens tend to be less immunogenic
## Some Viral Vaccines

<table>
<thead>
<tr>
<th>Disease</th>
<th>Vaccine</th>
<th>Recommendation</th>
<th>Booster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza</td>
<td>Injected vaccine, inactivated virus (nasally administered vaccine with attenuated virus is now available for some)</td>
<td>For chronically ill, including children over 6 months. Adults over age 65. Healthy children aged 6–23 months (because higher risk of related hospitalizations). Health care workers and others in contact with high risk groups. Healthy persons aged 5–49 years can receive intranasal vaccine.</td>
<td>Annual</td>
</tr>
<tr>
<td>Measles</td>
<td>Attenuated virus</td>
<td>For infants age 15 months</td>
<td>See Table 18.3</td>
</tr>
<tr>
<td>Mumps</td>
<td>Attenuated virus</td>
<td>For infants age 15 months</td>
<td>(Duration of immunity not known)</td>
</tr>
<tr>
<td>Rubella</td>
<td>Attenuated virus</td>
<td>For infants age 15 months; for females of childbearing age who are not pregnant</td>
<td>(Duration of immunity not known)</td>
</tr>
<tr>
<td>Chickenpox</td>
<td>Attenuated virus</td>
<td>For infants age 12 months</td>
<td>(Duration of immunity not known)</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>Killed virus</td>
<td>For children, see Table 18.3; for adults, as risk to exposure warrants</td>
<td>(Duration of immunity not known)</td>
</tr>
<tr>
<td>Rabies</td>
<td>Killed virus</td>
<td>For field biologists in contact with wildlife in endemic areas; for veterinarians; for people exposed to rabies virus by bites</td>
<td>Every 2 years</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>Antigenic fragments of virus</td>
<td>For infants and children, see Table 18.3; for adults, especially health care workers, homosexual males, injecting drug users, heterosexual people with multiple partners, and household contacts of hepatitis B carriers</td>
<td>Duration of protection at least 7 years; need for boosters uncertain</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>Inactivated virus</td>
<td>Mostly for travel to endemic areas and protecting contacts during outbreaks</td>
<td>Duration of protection estimated at about 10 years</td>
</tr>
<tr>
<td>Smallpox</td>
<td>Live vaccinia virus</td>
<td>Certain military and health care personnel</td>
<td>Duration of protection estimated at about 3 to 5 years</td>
</tr>
</tbody>
</table>
2. Monoclonal vs Polyclonal Antibody
Polyclonal Antibodies

Collection of antibodies (Ab’s) produced by many B cells specific for the same antigen (i.e., from many B cell “clones”)

1) immunize animal (usu. rabbit, goat, chicken) w/desired antigen (protein or whole pathogen)

2) collect blood serum from immunized animal (full of Ab’s that bind various epitopes on antigen)

3) use for testing
Monoclonal Antibodies

A monoclonal Ab (mAb) is a collection of identical Ab’s from a single B cell clone.

Sometimes monoclonal Ab’s are preferable to polyclonal Ab’s

• much “cleaner” than polyclonal serum, can give cleaner, more precise results
  • recognize only 1 epitope on the antigen

• preferable when greater target specificity is needed
Many techniques require a monoclonal antibody (mAb), however B cells don’t grow in cell culture:

- B cells die after a few days in culture, they are not immortal like cancer cells
- it is necessary to somehow obtain an immortal B cell clone in order to have a steady supply of the mAb

So how can you get an immortal B cell clone?

- you combine the antibody production of a B cell with the immortality of a cancer cell line…
How are monoclonal Ab’s produced?

1) Immunize mouse with antigen

2) Induce the fusion of B cells from spleen of immunized mouse and immortal mouse myeloma cells (cancerous)
3) select for B cell hybridomas
   • due to fusion of B cell and myeloma cell

Mix and fuse cells

grow in selective medium that will kill myeloma cells (unfused B cells die on their own)

Unfused cells (● ● ● ●) die
Fused cells (○ ○ ○ ○) grow
4) identify hybridomas producing antibody to antigen
   • test for binding to desired antigen
Summary of monoclonal Ab production

1) immunize mouse w/desired antigen

2) fuse mouse splenocytes (rich in B cells) with modified myeloma cell line (immortal but can’t survive in selective medium)

3) select for hybridomas (B cell/myeloma fusions) by growth in selective medium
   • unfused myeloma cells can’t grow, unfused B cells don’t survive for very long (not immortal)
   • hybridomas are immortal, resistant & make Ab!

4) screen for hybridomas that produce desired Ab
   • test with desired antigen
3. Diagnostic Immunology
Techniques in Diagnostic Immunology

Diagnostic immunology involves using antibodies to acquire clinical data using procedures such as:

Precipitation Reactions
  - the formation of insoluble Ab:Ag complexes

Agglutination
  - the formation of visible Ab:Ag aggregates

Neutralization Reactions
  - inhibition of cytopathic effects due to antibody binding

Fluorescent Antibody Staining
  - reveal the presence of specific pathogens

ELISA
  - automated technique revealing presence of Ab or Ag
The Nature of Immunoprecipitation

Soluble protein antigen and antibody will form insoluble complexes when mixed in the right proportions:

- Excess antibody or antigen will result in no insoluble material.
- Equal proportions of antibody & antigen result in an insoluble complex (precipitate) of interconnected antibody complexes.

**usually works only with polyclonal antibody**
The Precipitin Ring Test

The presence of specific antigen or antibodies in a test sample (e.g., blood serum) can be revealed by layering a solution of one on top of the other:

- at the interface between antigen & antibody, the two will mix due to diffusion
- within this region of mixing a “zone of equivalence” will form (equal amounts of Ag & Ab)
- this results in a visible ring of precipitation which indicates a positive result
Direct Agglutination Test

Large, complex antigens (e.g., viruses or bacteria) can be agglutinated by specific antibody:

- does not require precise proportions as with immuno-precipitation
- can use polyclonal or monoclonal antibody
- allows detection of antibody to specific antigens as well as the determination of “antibody titer” by serial dilution

Copyright © 2007 Pearson Education, Inc.
Indirect Agglutination

Involves the same basic principles as with the direct agglutination, except uses multiple copies of specific protein antigen or antibody attached to a synthetic particle (i.e., a “bead”):

• agglutination occurs due to multiple copies of epitope or Ab on same particle

(a) Reaction in a positive indirect test for antibodies. When particles 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 antigens.
Many viruses such as influenza virus can stick to and agglutinate red blood cells in a process called **viral hemagglutination**:

- does not involve any antibodies yet works in the same manner
Neutralization of Viral Hemagglutination

This type of diagnostic test reveals the presence of specific viral antibodies in serum (i.e., due to exposure to the virus) due to the prevention of viral hemagglutination:

- antibodies to the virus in serum (if present) will inhibit hemagglutination by binding to virus
Neutralization of Bacterial Toxins

Bacterial toxins can also be effectively neutralized by specific antibodies:

- in vitro tests of this type are useful for detecting either the presence of toxin OR antibody specific for the toxin.
Direct Fluorescent Antibody Labeling

Antibodies labeled with a fluorescent dye are useful for identifying pathogens in a tissue sample for example:

- with direct labeling the primary antibody (antibody that binds to the pathogen) itself is labeled.

(a) Reactions in a positive direct fluorescent-antibody test
Indirect Fluorescent Antibody Labeling

It is frequently more practical to label a secondary antibody ($2^\circ \text{Ab}$) to reveal the binding of unlabeled primary antibody ($1^\circ \text{Ab}$)

- this indirect method is useful for detection the presence of specific antibody in clinical samples
- $2^\circ \text{Ab}$ is specific for constant region of $1^\circ \text{Ab}$

(b) Reactions in a positive indirect fluorescent-antibody test

T. pallidum from laboratory stock

Specific antibodies in serum of patient

Antibodies bind to T. pallidum

Fluorescent dye-labeled anti-human immune serum globulin (This will react with any immunoglobulin)

Fluorescent spirochetes

Copyright © 2007 Pearson Education, Inc., publishing as Benjamin Cummings.
ELISA

ELISA stands for Enzyme-Linked Immunosorbent Assay and has the following features:

- it involves the use of multi-well plates and automated plate readers
  - allows the rapid analysis of large numbers of samples
- uses antibodies labeled with a specific enzyme
  - addition of enzyme substrate results in colored product that is visible and measurable by plate reader
- can be used to detect the presence of Ab or Ag
  - *antigen* is fixed to a surface to detect *antibody*
  - *antibody* is fixed to a surface to detect *antigen*
• with direct ELISA a labeled 1° Ab is used to detect antigen
Indirect ELISA

1. Antigen is adsorbed to well.

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

3. Enzyme-linked anti-HISG (see page 543) is added and binds to bound antibody.

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

- With indirect ELISA a labeled 2° Ab is used to detect 1° Ab.
Key Terms for Chapter 18

• attenuated vs inactivated whole agent vaccines
• toxoid, subunit & conjugated vaccines
• hapten
• monoclonal vs polyclonal antibody
• immunoprecipitation, precipitin ring test
• direct vs indirect agglutination
• viral hemagglutination
• direct vs indirect ELISA

Relevant Chapter Questions
rvw: 1-5, 7-11  MC: 1-5, 7-10