Antibody production

Primary immunization with different doses of antigen

Primary response

Secondary immunization with single antigen doses of $10^3$

Secondary response

Antibody response (arbitrary units)

Antigen dose

Figure A-1 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)
Inducing B and T cell responses

1. **B cell binds virus through viral coat protein**
   - Epitope

2. **Virus particle is internalized and degraded**

3. **Peptides from internal proteins of the virus are presented to the T cell, which activates the B cell**
   - CD154 (CD40L)
   - CD40
   - Cytokines

4. **Activated B cell produces antibody against viral coat protein**

*Figure 9-3 Immunobiology, 6/e. (© Garland Science 2005)*
Types of antisera

• Polyclonal=
  – Collection of antibodies from the serum
  – Multiple binding specificities to many epitopes of one or more antigens

• Monoclonal=
  – Homogeneous preparation of antibody
  – Single binding specificity to one epitope on a single antigen
Direct vs. indirect assays: (the immunological definition)

• Direct assays = detect Ag using a known Ab

• Indirect assays = detect Ab to a known Ag
  – In clinical setting, suggest present or past exposure -> seroconversion

• Titer = qualitative amount of antibody in the serum
  – Reciprocal of dilution that yields a positive response in test
Uses of immunological techniques

• Clinical applications
  – Detection of specific pathogen
  – Detection of antibody to specific pathogen
    • Seroconversion
    • Potential vaccine candidates
  – Detection of antibody to self: Autoimmunity
Uses of immunological techniques

• Research applications
  – Size of protein of interest
  – Localization of protein within a cell
  – Tissue distribution of protein
  – Levels of protein expression under different conditions
  – Modification of protein
  – Identification of protein partners
Agglutination reaction

- Mixture of antigen and antigen
- Visible precipitation reaction
- Quick, but not very sensitive
Agglutination reactions

<table>
<thead>
<tr>
<th>Serum from individuals of type</th>
<th>O - GlcNAc - Gal/Fuc</th>
<th>A - GlcNAc - Gal - GlcNAc/Fuc</th>
<th>B - GlcNAc - Gal/Fuc</th>
<th>AB - GlcNAc - Gal - GlcNAc/Fuc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A and anti-B antibodies</td>
<td>no agglutination</td>
<td>agglutination</td>
<td>agglutination</td>
<td>agglutination</td>
</tr>
<tr>
<td>Anti-B antibodies</td>
<td>no agglutination</td>
<td>no agglutination</td>
<td>agglutination</td>
<td>agglutination</td>
</tr>
<tr>
<td>Anti-A antibodies</td>
<td>no agglutination</td>
<td>agglutination</td>
<td>no agglutation</td>
<td>agglutation</td>
</tr>
<tr>
<td>No antibodies to A or B</td>
<td>no agglutination</td>
<td>no agglutation</td>
<td>no agglutation</td>
<td>no agglutation</td>
</tr>
</tbody>
</table>

Figure A-8 Immunobiology, 6/e. (© Garland Science 2005)
Enzyme-linked immunosorbent assay (ELISA)
ELISA in microplate assay
ELISPOT -measures T cell cytokine responses
ELISA/ELISPOT

- Very sensitive
- Best quantitative method for determining levels of antigen/antibody
Immunofluorescence
Immunofluorescence

Excitation and emission wavelengths of some commonly used fluorochromes

<table>
<thead>
<tr>
<th>Probe</th>
<th>Excitation (nm)</th>
<th>Emission (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-phycoerythrin (PE)</td>
<td>480; 565</td>
<td>578</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>495</td>
<td>519</td>
</tr>
<tr>
<td>PerCP</td>
<td>490</td>
<td>675</td>
</tr>
<tr>
<td>Texas Red</td>
<td>589</td>
<td>615</td>
</tr>
<tr>
<td>Rhodamine</td>
<td>550</td>
<td>573</td>
</tr>
</tbody>
</table>
Immunofluorescence

• Can provide information about localization of protein within cells or tissues
• Semi-quantitative, Western blotting or ELISAs used for more quantitation of protein levels
Western blotting
Western blotting

• Can provide:
  – Size of protein of interest
  – Tissue distribution
  – Species distribution
  – Levels of protein of interest (not as easy to quantitate as ELISA)
Affinity purification

- Purification of:
  - antigen specific to column-bound antibody
  - antibody specific to column-bound antigen
Expression libraries

Identifying and isolating cDNA for:
- any gene of interest
- vaccine candidates
Immunizations

• In humans, inducing an active adaptive immune response to protect against pathogens= vaccination

• In animals,
  – Protection of animals
  – Antibody production for clinical/research use
Passive Immunization

• Providing antibodies to a recipient
• Not inducing an active adaptive response
• Examples:
  – Ig in breast milk, across placenta
  – Anti-toxin antibodies
    • Snake anti-venom
    • Tetanus treatment
Considerations that impact vaccine development and use:

- Immunological
- Safety
- Administration
- Delivery
- Health needs of community
- Financial

### Features of effective vaccines

<table>
<thead>
<tr>
<th>Safe</th>
<th>Vaccine must not itself cause illness or death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protective</td>
<td>Vaccine must protect against illness resulting from exposure to live pathogen</td>
</tr>
<tr>
<td>Gives sustained protection</td>
<td>Protection against illness must last for several years</td>
</tr>
<tr>
<td>Induces neutralizing antibody</td>
<td>Some pathogens (such as poliovirus) infect cells that cannot be replaced (e.g., neurons). Neutralizing antibody is essential to prevent infection of such cells</td>
</tr>
<tr>
<td>Induces protective T cells</td>
<td>Some pathogens, particularly intracellular, are more effectively dealt with by cell-mediated responses</td>
</tr>
<tr>
<td>Practical considerations</td>
<td>Low cost-per-dose Biological stability Ease of administration Fewside-effects</td>
</tr>
</tbody>
</table>

Fig 14.23 © 2001 Garland Science
Inducing B and T cell responses

B cell binds virus through viral coat protein

Virus particle is internalized and degraded

Peptides from internal proteins of the virus are presented to the T cell, which activates the B cell

CD154 (CD40L)

CD40

Helper T cell

cytokines

Activated B cell produces antibody against viral coat protein

Figure 9-3 Immunobiology, 6/e. (© Garland Science 2005)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Increased immunogenicity</th>
<th>Decreased immunogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>Large</td>
<td>Small (MW&lt;2500)</td>
</tr>
<tr>
<td>Dose</td>
<td>Intermediate</td>
<td>High or low</td>
</tr>
<tr>
<td>Route</td>
<td>Subcutaneous &gt; intraperitoneal &gt; intravenous or intragastric</td>
<td></td>
</tr>
<tr>
<td>Composition</td>
<td>Complex</td>
<td>Simple</td>
</tr>
<tr>
<td>Form</td>
<td>Particulate</td>
<td>Soluble</td>
</tr>
<tr>
<td></td>
<td>Denatured</td>
<td>Native</td>
</tr>
<tr>
<td>Similarity to self protein</td>
<td>Multiple differences</td>
<td>Few differences</td>
</tr>
<tr>
<td>Adjuvants</td>
<td>Slow release</td>
<td>Rapid release</td>
</tr>
<tr>
<td></td>
<td>Bacteria</td>
<td>No bacteria</td>
</tr>
<tr>
<td>Interaction with host MHC</td>
<td>Effective</td>
<td>Ineffective</td>
</tr>
</tbody>
</table>

Figure A-2 Immunobiology, 6/e. (© Garland Science 2005)
<table>
<thead>
<tr>
<th>Adjuvant name</th>
<th>Composition</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incomplete Freund's adjuvant</td>
<td>Oil-in-water emulsion</td>
<td>Delayed release of antigen; enhanced uptake by macrophages</td>
</tr>
<tr>
<td>Complete Freund's adjuvant</td>
<td>Oil-in-water emulsion with dead mycobacteria</td>
<td>Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators in macrophages</td>
</tr>
<tr>
<td>Freund's adjuvant with MDP</td>
<td>Oil-in-water emulsion with muramylidipeptide (MDP), a constituent of mycobacteria</td>
<td>Similar to complete Freund's adjuvant</td>
</tr>
<tr>
<td>Alum (aluminum hydroxide)</td>
<td>Aluminum hydroxide gel</td>
<td>Delayed release of antigen; enhanced macrophage uptake</td>
</tr>
<tr>
<td>Alum plus <em>Bordetella pertussis</em></td>
<td>Aluminum hydroxide gel with killed <em>B. pertussis</em></td>
<td>Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators</td>
</tr>
<tr>
<td>Immune stimulatory complexes (ISCOMs)</td>
<td>Matrix of Quil A containing viral proteins</td>
<td>Delivers antigen to cytosol; allows induction of cytotoxic T cells</td>
</tr>
</tbody>
</table>
Animal studies test efficacy of vaccine
Challenge experiment for heterotypic immunity

Vaccination strategy
- Vaccinated with formalin fixed H3N2 or H3N2 with *E coli* as adjuvant
- Intranasal or subcutaneous delivery
- Received 3 vaccinations

Challenge experiment
- Challenged 2 weeks later with H5N1
- Fig. A- survival after challenge
- Fig. B- amount of virus in tissue
<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Birth</th>
<th>1 month</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>12 months</th>
<th>15 months</th>
<th>18 months</th>
<th>24 months</th>
<th>4-6 years</th>
<th>11-12 years</th>
<th>13-18 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B¹</td>
<td>HepB #1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HepB Series</td>
<td></td>
</tr>
<tr>
<td>Diphtheria, Tetanus, Pertussis²</td>
<td>DTaP</td>
<td>DTaP</td>
<td>DTaP</td>
<td>DTaP</td>
<td>DTaP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DTaP</td>
<td>Td</td>
<td>Td</td>
</tr>
<tr>
<td>Haemophilus influenzae type b¹</td>
<td>Hib</td>
<td>Hib</td>
<td>Hib</td>
<td>Hib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inactivated Poliovirus</td>
<td>IPV</td>
<td>IPV</td>
<td>IPV</td>
<td>IPV</td>
<td>IPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measles, Mumps, Rubella⁴</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MMR #1</td>
<td></td>
<td></td>
<td></td>
<td>MMR #2</td>
<td>MMR #2</td>
<td></td>
</tr>
<tr>
<td>Varicella¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Varicella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV ²</td>
<td>PCV</td>
<td>PCV</td>
<td>PCV</td>
<td>PCV</td>
<td>PCV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PCV</td>
<td>PPV</td>
<td></td>
</tr>
<tr>
<td>Influenza†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Influenza (Yearly)</td>
<td>Influenza (Yearly)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis A¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of December 1, 2004, for children through age 18 years. Any dose not administered at the recommended age should be administered at any subsequent visit when indicated and feasible.

Indicates age groups that warrant special effort to administer those vaccines not previously administered. Additional vaccines may be licensed and recommended during the year. Licensed combination vaccines may be used whenever any components of the combination are indicated and other components of the vaccine are not contraindicated. Providers should consult the manufacturers' package inserts for detailed recommendations. Clinically significant adverse events that follow immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS). Guidance about how to obtain and complete a VAERS form is available at www.vaers.org or by telephone, 800-822-7967.

Range of recommended ages
Only if mother HBsAg(−)
Pedoadolescent assessment
Catch-up immunization
Types of vaccines

- Inactivated
- Attenuated
- Acellular
- Toxoid
- Conjugated
- Subunit
- DNA vaccines
Inactivated vaccines

• Killed, inactivated pathogen is injected
• Examples:
  • Rabies
  • Sabin polio/IPV
  • Hepatitis A
• Advantages and disadvantages?
Attenuated vaccines

• Live, but non-virulent pathogen injected

• Examples:
  • Salk polio vaccine (OPV)
  • Live attenuated influenza vaccine (FluMist)
  • Chickenpox
  • Measles/mumps/rubella (MMR)

• Advantages and disadvantages?
The pathogenic virus is isolated from a patient and grown in human cultured cells.

The cultured virus is used to infect monkey cells.

The virus acquires many mutations that allow it to grow well in monkey cells.

The virus no longer grows well in human cells (it is attenuated) and can be used as a vaccine.

Isolate pathogenic virus

Isolate virulence gene

Receptor-binding protein
Virulence
Core proteins

Mutate virulence gene
Delete virulence gene

Resulting virus is viable, immunogenic but avirulent. It can be used as a vaccine.
Attenuated vs. killed influenza vaccines

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>LAIV</th>
<th>Inactivated influenza vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of administration</td>
<td>Intranasal spray</td>
<td>Intramuscular injection</td>
</tr>
<tr>
<td>Type of vaccine</td>
<td>Live virus</td>
<td>Killed virus</td>
</tr>
<tr>
<td>Number of included virus strains</td>
<td>3 (2 influenza A, 1 influenza B)</td>
<td>Same as LAIV</td>
</tr>
<tr>
<td>Vaccine virus strains updated</td>
<td>Annually</td>
<td>Same as LAIV</td>
</tr>
<tr>
<td>Frequency of administration</td>
<td>Annually</td>
<td>Same as LAIV</td>
</tr>
<tr>
<td>Can be administered to children and adults at high risk* for complications resulting from influenza infection</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Can be administered to family members or close contacts of immunosuppressed persons</td>
<td>Inactivated influenza vaccine preferred</td>
<td>Yes†</td>
</tr>
<tr>
<td>Can be administered to family members or close contacts of persons at high risk but who are immunocompetent</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Can be simultaneously administered with other vaccines</td>
<td>Yes§</td>
<td>Yes</td>
</tr>
<tr>
<td>If not simultaneously administered, can be administered within 4 weeks of another live vaccine</td>
<td>Prudent to space 4 weeks apart</td>
<td>Yes</td>
</tr>
<tr>
<td>If not simultaneously administered, can be administered within 4 weeks of an inactivated vaccine</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Populations at high risk from complications of influenza infection include persons aged ≥65 years; residents of nursing homes and other facilities that house persons with chronic medical conditions; adults and children with chronic disorders of the pulmonary or cardiovascular systems; adults and children with chronic metabolic diseases (including diabetes mellitus), renal dysfunction, hemoglobinopathies, or immunosuppression; children and adolescents receiving long-term aspirin therapy (at risk for developing Reye syndrome after wild-type influenza infection); and women who will be in the second or third trimester of pregnancy during influenza season.
†Immunosuppressed persons include, but are not limited to, those persons with human immunodeficiency virus, malignancy, or those receiving immunosuppressive therapies.
§No data are available regarding effect on safety or efficacy.
¶Inactivated influenza vaccine coadministration with pneumococcal polysaccharide vaccine has been evaluated systematically only among adults.
Acellular vaccine

- Components from pathogen purified and injected

- Examples:
  - Pertussis (whooping cough)

- Advantages/disadvantages?
Toxoid vaccines

• Inactivated toxin is injected

• Examples:
  • Tetanus
  • Diptheria

• Advantages/disadvantages?
Conjugate vaccines

• Two substance are administered, one to increase the immunogenicity of the other

• Examples:
  • Haemophilus influenzae B (HiB) vaccine
    – Polysaccharides of H. influenzae attached to:
      » Diptheria toxoid
      » Tetanus toxoid
      » Meningococcal outer membrane protein
  • Pneumococcus
Conjugate vaccine strategy

- B cell binds bacterial polysaccharide epitope linked to tetanus toxoid protein
- Antigen is internalized and processed
- Peptides from protein component are presented to the T cell
- Activated B cell produces antibody against polysaccharide antigen on the surface of the bacterium

Figure 9-4 Immunobiology, 6/e. (© Garland Science 2005)
Subunit vaccine

• Protein from pathogen is expressed in heterologous system (bacteria/yeast), purified and injected

• Examples:
  • Hepatitis B

• Advantages/disadvantages?
DNA vaccines

• Plasmid DNA encoding virulence gene is injected

• Some in clinical trials

• Advantages/disadvantages?
DNA vaccine method

Clone gene for influenza hemagglutinin in a plasmid

Inject cloned gene into muscle tissue

Infect mice with influenza virus

Measure virus titer

Figure 14-28 Immunobiology, 6/e. (© Garland Science 2005)