Vaccines

BIT 120
Immunization

- **Immunization**: a procedure designed to increase concentrations of antibodies and/or effector T-cells which are reactive against infection (or cancer).

- Immunization procedure called vaccination and the immunizing agent called vaccine (or “serum” in historical references)
Immunization (cont’d)

• When performed before exposure to an infectious agent (or soon after exposure in certain cases), it is called **immunoprophylaxis**, intended to *prevent* the infection.

• When performed during an active infection (or existing cancer), it is called **immunotherapy**, intending to *cure* the infection (or cancer)
Types of Immunity

- Two mechanisms by which immunization can be achieved
- Passive immunization:
  - Protective Abs --> non immune recipient
  - No immunological memory w/o Th cells.
- Active immunization:
  - Induction of adaptive immune response, with protection and memory.
Passive and Active Immunization

**TYPE ACQUIRED THROUGH Passive Immunization –**
Natural maternal serum/milk
Artificial immune serum

**Type ACQUIRED THROUGH Active Immunization –**
Natural infection
Artificial infection*:
  - Attenuated organisms (live)
  - Inactivated organisms (dead)
  - Cloned genes of microbiological antigens
  - Purified microbial macromolecules
  - Synthetic peptides
  - DNA

*Artificial refers to steps involving human intervention
Passive Immunity

**Naturally** - transplacental transfer of maternal IgG Abs to developing fetus; transfer of IgG + IgA Abs in milk during breast-feeding of newborn

**Medically** - injection of immune globulin  Performed prophylactically, either after diagnosis of exposure to toxin/virus or as a short term preventive procedure, e.g. if one is traveling to an endemic area

**Blocking** - prevent hemolytic anemia of the newborn: Rhogam injected into pregnant Rh- mother prior to delivery of each baby conceived with Rh+ father.
Active Immunization

**Naturally** - following exposure to an infection

**Medically** - by vaccination: Performed either by i.m. injection of killed or attenuated antigens (often with adjuvant) or by ingestion of attenuated live organisms.

**Blocking** - Reversal of auto-immune response

**Anti-cancer** - Reactivation of tumor-stimulated T lymphocytes.
Mechanism of Vaccination

Establish resistance to virus/pathological organism by evoking an immune response

1. Give host a foreign organism/protein in non-infectious form

2. Antibodies are generated
   Ab binds to surface proteins of organism
Structure of a Virus particle
I. Types
   A. Inactivated (Killed)
   B. Live
   C. Attenuated (Live, Non-infectious)

   LIVE MORE EFFECTIVE THAN KILLED

II. Pathogens
   A. Bacteria
   B. Virus
   C. Parasites
Types of Vaccines

Attenuated – live microbe (usually virus) which has a vital function inactivated by heat, chemicals or genetic manipulation

- e.g. Rabies virus vaccine, MMR (Measles, Mumps and Rubella)
- BCG (Bacillus Calmette Guerin vaccine for *Mycobacterium tuberculosis*)
- Risk it could revert back to infectious agent
- will stimulate both cell mediated and antibody mediated immune responses
Types of Vaccines (cont’d)

• **Inactivated** – uses toxoid – inactivated toxins which are purified proteins
  – stimulates the antibody mediated response only
  – e.g. DPT (diphtheria, pertussis, tetanus toxoids)
  – stimulates the antibody mediated response only
Types of Vaccines (cont’d)

• **Component (subunit)** – contains purified components from bacteria and viruses

• How recombinant viruses are made
  – Hepatitis B vaccine – purified viral coat protein
  – *Streptococcus pneumoniae* (PneumoShot) – capsular polysaccharide
  – *Hemophilus influenzae* (HiB) – capsular polysaccharide, part of DPTPolio-
  – Hib vaccine given to infants
  – *Neisseria meningitidis* – capsular polysaccharide
  – stimulates the antibody mediated response only
Other vaccinations/components

- **Booster Shots**: same vaccine given at a later date (e.g. DT given every 10 years
  - to refresh the memory cell population

- **Adjuvant**: chemicals in the vaccine solution that enhance the immune response
  - Alum – Ag in the vaccine clumps with the alum such that the Ag is released
  - slowly, like a time-release capsule
  - gives more time for memory cells to form
Antibody Titer

• A test to measures the presence and amount of antibodies in blood against a particular type of tissue, cell, or substance
• Titer determines if you have adequate protection against a disease
• May need to give booster if titer too low
• E.g., happens with HepB vaccine
Herd Immunity

• Indirect protection from infection among susceptible members of a population, and the protection of the population as a whole, due to the presence of the immune individuals

• Therefore, leads to reduction of transmission in a population (sometimes can lead to the disappearance of the disease)
Herd Immunity Animation

- [http://www.immunisation.nhs.uk/herdimm02.html](http://www.immunisation.nhs.uk/herdimm02.html)

- See text next slide
Scene 1:
A population without an immunization program against a disease is vulnerable to all the effects of that disease. Many people will suffer, some die, some may be left with a permanent disability.

Scene 2:
Even if there is a vaccination program, if not enough people get the vaccination, the germ can still infect those who aren't protected.

Scene 3:
When enough people are vaccinated, it is very hard for the germ to find anyone who isn't vaccinated. Because of this 'herd immunity', non-immunized people are protected. They are protected both from catching the disease and suffering any permanent disability.

Scene 4:
When enough people are protected, it's possible for some diseases to disappear forever, as happened with smallpox, and will happen with polio and eventually measles.
When this happens, the World Health Organization can certify the world to be free of that disease and vaccination for that disease will no longer be needed.
## Common Vaccinations in Infants and Children

**Also See Handout**

<table>
<thead>
<tr>
<th>AGE</th>
<th>VACCINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth--2 mos.</td>
<td>Hepatitis B (recombinant surface Ag)</td>
</tr>
<tr>
<td>2 mos.</td>
<td>Diptheria, tetanus, acellular pertussis (DTaP)</td>
</tr>
<tr>
<td></td>
<td>Inactivated Polio virus (IPV)</td>
</tr>
<tr>
<td></td>
<td>Haemophilus influenzae b (Hib-conjugate)</td>
</tr>
<tr>
<td>2-4 mos.</td>
<td>Hep B</td>
</tr>
<tr>
<td>4 mos.</td>
<td>DTaP, IPV, Hib, PCV</td>
</tr>
<tr>
<td>6-18 mos.</td>
<td>Hep B, DTaP, Polio (IPV), Hib</td>
</tr>
<tr>
<td>12-15 mos.</td>
<td>DTaP, Hib</td>
</tr>
<tr>
<td></td>
<td>Varicella zoster/chicken pox (VZV)</td>
</tr>
<tr>
<td></td>
<td>Measles, mumps, rubella (MMR)</td>
</tr>
<tr>
<td>4-6 yrs</td>
<td>DTaP, IPV, MMR</td>
</tr>
<tr>
<td>11-12 yrs</td>
<td>DT</td>
</tr>
</tbody>
</table>

**SOURCE:** Centers for Disease Control
Limitations To Traditional Vaccines

1. can’t grow all organisms in culture

2. safety to lab personnel

3. Expense

4. insufficient attentuation

5. reversion to infectious state

6. need refrigeration

7. do not work for all infectious agents

8. infants/children receive them – immature immunity
Why are antibiotics not good enough?

1. Antibiotic-resistance

2. Need refrigeration

3. People do NOT take entire regiment – therefore better always to prevent disease
Recombinant Vaccines

1. Subunit Vaccines
   peptide vaccines
   Genetic immunization

3. Attenuated Vaccines

4. Vector Vaccines

5. Bacterial Antigen Delivery Systems
Recombinant Vaccines

1. Delete Virulence Genes (can not revert)
   V/B as Vaccine

2. Clone gene for pathogenic antigen into non-pathogenic virus or bacteria
   V/B as Vaccine

3. Clone pathogenic antigen gene into expression vector

   A. Vaccinate with ‘protein’
      1. Subunit
      2. Peptide
Subunit vaccines

- Do NOT use entire virus or bacteria (pathogenic agent)
- Use components of pathogenic organism instead of whole organism
- Advantage: no extraneous pathogenic particles ie DNA
- Disadvantage: Is protein same as *in situ*?
  Cost
Examples of Subunit Vaccines

A. HSV

• Problem with Traditional vaccine- HSV is oncogenic

• Envelope glycoprotein D (gD) elicits Ab response

• Clone gene for gD into vector
  • Express in mammalian cells

• Transmembrane protein
  • Modify gene to remove TM portion
Other Subunit Vaccines

B. Tuberculosis

\textit{Mycobacterium tuberculosis}

antibiotic resistant strains

use purified extracellular (secreted) proteins as Vaccine

C. Foot-and-Mouth Disease virus

cattle/pigs

VP1 capsid viral protein elicits response -
used this protein as Vaccine
Peptide Vaccines

Use discrete portion (domain) of a surface protein as Vaccine

These domains are ‘epitopes’

antigenic determinants
are recognized by antibodies
CARRIER PROTEINS – help vaccine production

Small Peptides are often Digested

Carrier Proteins Make more Stable
FMDV peptide vaccine

Problem:
Large quantities of peptide needed to be used to get immunological response

Solution:
Use highly immunogenic carrier molecule

HBcAg was a suitable carrier
(Hepatitis Core Protein)

Fused peptide DNA with gene for HBcAg

This fusion protein used as Vaccine
Genetic Immunization

Delivery of a gene for the antigen to a host organism

Use vector containing cDNA from viral protein/eukaryotic promoter

Inject into muscle/microprojectile system

POTENTIAL
- eliminates purification of antigen
- protein is modified post-translationally

FATE of plasmid DNA
- integration?
- degradation?
Attenuated vaccines

Cholera
- caused by bacterium
- lives in intestine causing diarrhea, dehydration
- poor sanitation (water supply, sewage)
- secretes an enterotoxin (A1) which causes disease
- killed vaccine not effective long-term
- subunit not effective

Phenol-killed cholera used as vaccine currently

Generally double deletions are preferred
can not multiple in host
Cholera Vaccines

A. Insert tetracycline gene into bacterium’s host chromosome
   This gene interrupts A1 peptide gene
   (toxic portion of the enterotoxin)

   NOT ACCEPTABLE
   Reversion by spontaneous excision

B. Deleted A1 peptide sequence created
Plasmid will eventually be lost

Bacterium will be tet sensitive

550 bp removed
Vector Vaccines: Virus as Antigen Gene Delivery System

Antigen Gene

\[ \downarrow \]

Virus

\[ \downarrow \]

Patient  \[ \rightarrow \]  Antigen Protein is Made
Vector vaccines

Vaccinia good candidate for a live recombinant viral vaccine
• benign virus
• replicate in cytoplasm (viral replication genes)
• easy to store

A) Insert cloned gene encoding antigen
B) Interrupt thymidine kinase (non-essential gene)
C. Infect host cell with native virus
D) Transform these cells with recombinant plasmid
E) HOMOLOGOUS RECOMBINATION
F) Select cells which are resistant to BROMODEOXYURIDINE

**MODIFIED VIRUS USED AS VACCINE**
Can insert more than one antigen gene
Control of Viral Vaccines Post Innoculation

• Vaccinia virus is resistant to interferon presence of K3L protein

• Use an interferon-sensitive strain of vaccinia virus
  • delete K3L gene to create mutant
Bacterial Antigen Delivery Systems: Bacterial Vectors

Antigen Gene

Bacterium

Antigen Proteins made on Bacterial cell

Vaccinate Patient
Bacterial Antigen Delivery Systems

• Use live nonpathogenic bacterium which contains antigen
  (Salmonella) (epitope from cholera)

• Insert antigen gene into flagellin gene

• Epitope is expressed on the flagellum surface

***Flagellin-engineered bacteria is VACCINE***

Advantage - Oral Administration
Does Immunologic Memory = Immune Protection???

• For protection by Abs- sufficiently high level of circulating Abs have to be present during challenge.
• For protection by T cells-a sufficient number of effectors have to be present or have to be generated soon after exposure (consider the pace of infection and presence of Abs)
“Memories” are made of these

• Increase in frequency of Ag-reactive cells

• Increase in reactivity of effector/memory cells
Vaccine Approval

- Done by CBER (Center for Biologics Evaluation and Research), an arm of the FDA
- Generally same clinical trial evaluation as other biologics and drugs
- Site to learn more about vaccines:

http://www.fda.gov/cber/vaccine/vacappr.htm