Chapter 17

Viral Vaccines
• Jenner, 1796
• Pasteur, 1885 – rabies vaccine; introduced the term vaccination from vacca (cow) in honor of Jenner
• Yellow fever, influenza vaccines – 1930’s
Immune induction and vaccine efficacy

SIDEBAR 17.1
Principles of immune induction relevant for vaccine efficacy

- Immune induction is much more efficient if an immunogen is presented by professional APCs, such as macrophages and dendritic cells.
- There is a relationship between the amount of antigen presented and the number of naïve lymphocytes that are induced to respond. The number of T lymphocytes induced during the active response determines the number of antigen-specific memory T lymphocytes that are generated.
- Following immune induction about 10–15 cell divisions occur in antigen-responsive T lymphocytes at which time there is no further proliferation. After a ‘rest’ of weeks to months, antigen-committed T cells may then be induced to proliferate again, to produce an anamnestic immune response.

- For many viral infections, both immunoglobulin and cellular effector systems can participate in protective immunity, but their relative role varies for different viruses.
- Immune induction can be manipulated to favor either T_H1 (cellular) or T_H2 (antibody) responses, by formulation of immunogen, route of immunization, and the use of adjuvants.
- Adjuvants can enhance the immune response in a variety of ways, mediated by their induction of pro-inflammatory cytokines.
- Presentation of antigen to the mucosa-associated lymphoid system (MALT) can induce local immunity, which may provide an effective barrier to viruses that invade via mucosal tissues.
Figure 8-46 The Immune System, 2/e (© Garland Science 2005)
Vaccines – stimulate immunological memory

- 1781 – outbreak of measles on Faroe Islands
- Next 65 years, island free of measles
- 1846 – another measles outbreak; none of those that survived the 1781 epidemic were infected.
- A ‘natural experiment’ demonstrating immune memory
- Immune memory lasts a long time and is maintained without re-exposure to virus
Smallpox - viral pathogen that has killed ~ 300 million People in the 20th century

Has been eradicated from human population through Vaccination

Numerous civilizations recognized that people that survived smallpox were extremely resistant to re-infection

Thus, the process of “variolation” - whereby scabs were collected from smallpox patients, dried, and inhaled or rubbed into open lesion of skin
Records from Sung Dynasty (China) from 960-1280 indicate that variolation was practiced in some form to protect people from smallpox.

During Revolutionary War, George Washington was so concerned that the British would use smallpox as a weapon that he had entire Continental Army variolated.
Edward Jenner - British physician noticed that many cowmaids had perfect skin

More importantly, recognized that those cowmaids that had been exposed to cowpox were protected from smallpox

Therefore, he reasoned that cowpox infection elicited some form of protection against infection by smallpox

Thus, the field of vaccination was developed whereby dried scabs from cowpox lesions were used to vaccinate people and this resulted in protection from disease
REPORTS
OF A
SERIES OF INOCULATIONS
FOR THE
VARIOLE VACCINE,
or
COW-POX;
WITH
REMARKS AND OBSERVATIONS ON THIS
DISEASE, CONSIDERED AS A SUBSTITUTE FOR
THE
SMALL-POX.

By WILLIAM WOODVILLE, M.D.
PHYSICIAN TO THE SMALL-POX AND INOCULATION HOSPITALS.

London:
Printed and Sold by
James Phillips and Son,
GEORGE-YARD, LOMBARD-STREET.
1799.
Jenner’s strategy - unique in that the pathogenic smallpox virus had a natural safe counterpart.
Large scale vaccines – are successful

Poliovirus vaccination in the USA

Inactivated vaccine (IPV) introduced
Oral, live-attenuated vaccine (OPV) introduced

Measles–United States, 1950-2001
Immune Memory

- Memory T and B cells are retained after infection
- Able to respond quickly after re-infection
- Vaccines establish immune memory without disease or first encounter with virus/bacteria
Requirements of a safe vaccine

- Safe – must not cause disease and have minimal side effects
- Must induce protective immunity in population
  - not every individual need to be immunized to stop viral spread
  - 80-95% immunity usually stops virus spread – herd immunity
- Protection must be long-lasting
- Low cost (<$1, WHO); genetic stability; storage considerations, delivery (oral vs needle).
### Approved human viral vaccines - 2006

<table>
<thead>
<tr>
<th>Date of approval USA</th>
<th>Virus and disease</th>
<th>Vaccine modality Route administration</th>
<th>Currently used in USA?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 1900</td>
<td>Variola Smallpox</td>
<td>Attenuated Intradermal</td>
<td>Only in the event of exposure</td>
</tr>
<tr>
<td>~1939</td>
<td>Yellow fever</td>
<td>Attenuated Subcutaneous</td>
<td>Only in the event of exposure</td>
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<tr>
<td>1955</td>
<td>Polio Poliomyelitis</td>
<td>Inactivated Intramuscular</td>
<td>Yes all infants</td>
</tr>
<tr>
<td>1963</td>
<td>Polio Poliomyelitis</td>
<td>Attenuated Oral</td>
<td>Yes special circumstances</td>
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<tr>
<td>1963</td>
<td>Measles</td>
<td>Attenuated Subcutaneous</td>
<td>Yes all infants</td>
</tr>
<tr>
<td>1967</td>
<td>Mumps</td>
<td>Attenuated Subcutaneous</td>
<td>Yes all infants</td>
</tr>
<tr>
<td>1969</td>
<td>Rubella German measles</td>
<td>Attenuated Subcutaneous</td>
<td>Yes all infants</td>
</tr>
<tr>
<td>1971</td>
<td>Influenza</td>
<td>Inactivated Intramuscular</td>
<td>Yes high risk only</td>
</tr>
<tr>
<td>1980</td>
<td>Rabies</td>
<td>Inactivated Intramuscular</td>
<td>Yes high risk only</td>
</tr>
<tr>
<td>1981</td>
<td>Hepatitis B</td>
<td>Inactivated Intramuscular</td>
<td>No no longer made</td>
</tr>
<tr>
<td>1986</td>
<td>Hepatitis B</td>
<td>Recombinant HBs protein Intramuscular</td>
<td>Yes all infants</td>
</tr>
<tr>
<td>1995</td>
<td>Varicella Chicken pox</td>
<td>Attenuated Subcutaneous</td>
<td>Yes all infants</td>
</tr>
<tr>
<td>~1996</td>
<td>Hepatitis A</td>
<td>Inactivated virus Intramuscular</td>
<td>Yes high risk only</td>
</tr>
<tr>
<td>2006</td>
<td>Rotavirus Infant diarrhea</td>
<td>Attenuated Oral</td>
<td>Yes Infants</td>
</tr>
</tbody>
</table>

# Types of Vaccines

## Advantages & Disadvantages

<table>
<thead>
<tr>
<th>Safety and efficacy</th>
<th>Advantages and disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Live attenuated viruses</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Safety</strong>&lt;br&gt;Advantages&lt;br&gt;None&lt;br&gt;&lt;br&gt;Disadvantages&lt;br&gt;Residual pathogenicity&lt;br&gt;Reversion to increased pathogenicity&lt;br&gt;Unrecognized adventitious agents&lt;br&gt;Possible persistence</td>
</tr>
<tr>
<td><strong>Efficacy</strong>&lt;br&gt;Advantages&lt;br&gt;Local immunity at portal of entry&lt;br&gt;Cellular and humoral immunity induction&lt;br&gt;Long-lasting immune response&lt;br&gt;Herd immunity&lt;br&gt;Less expensive to manufacture</td>
<td><strong>Disadvantages</strong>&lt;br&gt;Interference between serotypes&lt;br&gt;Interference by adventitious viruses&lt;br&gt;Loss of infectivity on storage&lt;br&gt;Cold chain required to maintain infectivity</td>
</tr>
</tbody>
</table>

| Inactivated or subunit viruses, or recombinant proteins | Safety | Advantages <br>Avoids dangers of attenuated viruses<br>Disadvantages <br>Potential residual infectious pathogenic virus<br>Safety tests difficult and expensive<br>Induction of unbalanced immune response |
| Efficacy | Advantages <br>No viral interference<br>Avoids limitations of attenuated viruses<br>Disadvantages <br>No induction of local immunity<br>Poor induction of cellular immunity<br>May not mimic native epitopes for humoral immunity<br>Short duration immunity (some products)<br>More expensive to manufacture |

**TABLE 17.1** The advantages and disadvantages of different vaccine modalities: attenuated viruses and non-replicating antigens
• Live-attenuated viruses - more potent in eliciting protective immune response due to ability to replicate to certain extent. Examples include: Measles, mumps, polio (Sabin), and Yellow fever
• Attenuation can be achieved by passage virus through cells from nonhuman species
Attenuated viruses

- Attenuated viruses produce infections that are milder than the illness produced by the counterpart virulent WT viruses.
- Often, attenuated viruses are host range mutants so their replicative capacity – relative to WT counterparts, is high in selected cell cultures but much lower in vitro.
- Attenuated vaccine viruses are selected for differential tropism in vivo compared to virulent parents.
- Oral poliovirus vaccine (OPV) replicates well in GI tract but poorly within the CNS.
- Usually, attenuated viruses are effective immunogens if they replicate effectively -> required for loading MHC class I and II molecules and stimulate B cell receptor/Ig binding.
- Immunogenicity decreases with reduction in *in vivo* replication.
- Some attenuated viruses retain residual pathogenicity. OPV causes occasional paralytic poliomyelitis (2/1x10^6 immunizations)

<table>
<thead>
<tr>
<th>Virus</th>
<th>Study period</th>
<th>Paralytic rate per 10^6 primary infections or immunizations</th>
<th>Relative rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wildtype</td>
<td>1931–1954</td>
<td>7000</td>
<td>~3000</td>
</tr>
<tr>
<td>OPV</td>
<td>1961–1978</td>
<td>2.3</td>
<td>1</td>
</tr>
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</table>
Attenuated viruses

• Some attenuated viruses may revert in virulence during passage in primary recipient which is a problem if virus is secreted. OPV often increases in virulence upon a single human passage due to revertant mutations and several small poliomyelitis outbreaks were traced to revertant strains.
• Inadvertant contamination with adventitious agents can be another problem with live virus vaccines. Yellow fever vaccine produced a massive epidemic of hepatitis B in the 1940s that was traced to a vaccine batch containing HBV.

**FIGURE 17.1** Live attenuated vaccine viruses may be inadvertently contaminated with extraneous infectious agents capable of causing disease. In this example, a batch of yellow fever virus was contaminated with HBV introduced with human serum used to ‘stabilize’ the yellow fever vaccine. Investigation showed that one of the serum donors was an asymptomatic carrier of HBV. The graph shows cases tabulated by weeks from immunization with 17D yellow fever vaccine to onset of jaundice. After Sawyer WA, MeyerKF, Eaton MD, Bauer JH, Putnam P, Schwentker FF. Jaundice in army personnel in the western region of the United States and its relation to vaccination against yellow fever. *American Journal of Hygiene* 1944, 39: 337–387.
Additional Strategies

• Innactivated viruses – chemical inactivation can inactivate viruses without destroying immunogenicity. Overall efficacy tends to be not as effective as attenuated viruses as generation of cellular immunity not as efficient.

• Recombinant proteins – Preparation of recombinant viral protein can be used to induce long-lasting immunity to some viral pathogens e.g. HBsAg (envelope protein of Hepatitis B virus).

• Recombinant viruses – deletion/addition of gene(s) that limit pathogenicity but regain immunogenicity.
Additional Strategies - Replicons

- Virus-like particles that will enter a target cell, undergo limited transcription and translation to generate an encoded protein(s) but will not produce infectious progeny. Therefore, unlike recombinant viruses, replicons can not spread.

- Replicons consist of a virus genome that has been engineered to insert a new protein and to delete some of the genes of the parent virus.

- Drawback is that the efficacy depends upon the number of target cells that are initially infected to produce enough novel immunogen to be delivered to APCs.

- Also, need to pass safety test to ensure they will not recombine with cellular sequences to reconstitute the potentially pathogenic viruses from which derived.
Additional Strategies
DNA – based immunogens

• DNA plasmid encoding a protein could be used as an immunogen by simple injection of naked DNA.
• DNA vaccine plasmids use a promoter that is highly active in eukaryotic cells that ensures expression.
• Modifications of the protein sequence can be used to influence how the protein is processed in APCs.
• Administered via intramuscular injection. To be immunogenic, DNA-encoded protein must be presented by professional APCs.
• Advantage is pre-existing immunity to DNA not a concern and avoids dangers intrinsic in attenuated or recombinant viruses.
• Drawbacks include generating sufficient amount of immunogen to induce effective immune response.
Protective Mechanisms by Established Vaccines

Poliovirus – pathogenesis and protection. Ab is important in providing protection following infection with PV.
OPV versus IPV

OPV provides induces better mucosal immunity compared to IPV.

**FIGURE 17.4** OPV (oral poliovirus vaccine) provides greater protection against enteric infection than does IPV (inactivated poliovirus vaccine). Three groups of children (naïve unimmunized; immunized with OPV; immunized with IPV) were tested for fecal excretion after feeding of OPV. Since the serum antibody levels were similar in the OPV and the IPV immunized groups, it was inferred that OPV induced local mucosal immunity more effectively than did IPV. After Henry JL, Jalkara ES, Davies JR *et al.* A study of poliovaccination in infancy: excretion following challenge with live virus by children given killed or living poliovaccine. *Journal of Hygiene* 1966, 64: 105–120, with permission.
Rabies virus and protective Ab

<table>
<thead>
<tr>
<th>Immunization status</th>
<th>Neutralizing antibody titer at challenge</th>
<th>Mortality dead/total</th>
<th>Mortality percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimmunized</td>
<td>&lt;2</td>
<td>17/17</td>
<td>82</td>
</tr>
<tr>
<td>Immunized</td>
<td>&lt;2</td>
<td>8/10</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3–9</td>
<td>2/5</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>10–99</td>
<td>4/18</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>100–999</td>
<td>0/21</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&gt;1000</td>
<td>1/13</td>
<td>8</td>
</tr>
</tbody>
</table>

**TABLE 17.7** Protection against rabies conferred by pre-exposure immunization correlates with neutralizing antibody levels at the time of challenge. Monkeys were immunized with rabies vaccine and were then challenged intramuscularly with $10^5$ mouse ic LD50 of street rabies virus. After Sikes RK, Cleary WF, Koprowski H, Wiktor TJ, Kaplan MM. Effective protection of monkeys against death from street virus by post-exposure administration of tissue-culture rabies vaccine. *Bulletin of the World Health Organization* 1971, 45: 10, with permission.
AIDS Vaccine

• HIV identified as cause of AIDS in 1983/84 yet no effective vaccine developed.
• Natural infection with HIV or immunization with recombinant gp120 induces Abs yet these have little or no ability to neutralize virus.
• Recombinant gp120 lacks ability to form trimers (naturally occurring form of gp120) and thus lacks conformational neutralizing epitopes.
• Attenuated viruses not yet generated/isolated that provide long-lasting protection.
Can HIV vaccine produce long-lasting immunity?

**Figure 17.10** The dynamics of HIV infection, illustrating the challenges to a prophylactic vaccine. Most viral vaccines are directed against acute infections and an immunized subject is successfully protected if the extent of replication is reduced for a period of days or a few weeks. However, reduction of acute HIV infection will not prevent the establishment of a persistent infection. In contrast to many other persistent infections that can be innocuous, HIV may eventually erode the CD4 lymphocyte population sufficiently to produce immunodeficiency, albeit with an extended incubation period.
Constant evolution of HIV results in changing epitopes and escape from neutralization

<table>
<thead>
<tr>
<th>Virus isolate months after infection</th>
<th>0 months</th>
<th>6 months</th>
<th>12 months</th>
<th>18 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;100</td>
<td>675</td>
<td>2670</td>
<td>2190</td>
</tr>
<tr>
<td>6</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>1769</td>
<td>2247</td>
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<tr>
<td>12</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>556</td>
</tr>
<tr>
<td>18</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>117</td>
<td>122</td>
</tr>
</tbody>
</table>

**Table 17.10** HIV-1 constantly evolves to escape neutralization by the patient's antibodies. In this example, virus isolates from a single patient were tested in a checkerboard against sera from the same patient. The patient did produce neutralizing antibodies but their activity was limited to virus isolates obtained at prior time points.

Protection against SHIV (simian HIV) in macaques by a prime-boost mixed modality vaccine. Animals were immunized at 0 and 2 months with DNA construct expressing most proteins of SHIV and were boosted at 6 months with recombinant vaccinia virus expressing major proteins (gag, pol, and env). At 13 months, animals were challenged by pathogenic SHIV. Viremia is controlled in vaccinated monkeys but not in control monkeys.
Candidate AIDS Vaccines

Partial protection of monkeys against virulent SIV with an immunogen containing gag, rev, tat, and nef genes. Formulated as a recombinant DNA vaccine and recombinant adenovirus followed by challenge with SIV 50 weeks post-immunization. Vaccinated animals exhibit both lower plasma virus and well-preserved CD4+ T cells.
Reprise

1. attenuated viruses – such as vaccinia or other poxviruses that have been engineered to express additional viral proteins
2. viral replicons, which are non-replicating constructs that will introduce either RNA or DNA encoding viral proteins into host cells
3. ‘naked’ DNA plasmids that encode a protein immunogen under the control of specific promoter sequences.