Vaccines

Lecture 20
Virology W3310/4310
Spring 2012

Nothing shocks me. I’m a scientist.
INDIANA JONES
By definition, viruses remain “one step ahead” of us

*Can we gain the upper hand?*

*Can we aid our natural defenses?*

*Can we remove a virus from the planet by designed intervention?*

The next two lectures will deal with our attempts to intervene in virus-host interactions with vaccines and antiviral drugs.
Two common threads connect the world of vaccines and antiviral drugs

1. Any agent or method that blocks virus replication or reduces viral pathogenesis imposes selections for virus mutants capable of bypassing the agent
   - Resistant mutants
   - Vaccine escape mutants
   - Selection for increased virulence

2. Viruses are obligate intracellular parasites; any intervention in the host-virus relationship carries inherent risks for the host
   - Those risks are the well-known side effects of therapy
Vaccines are our proven best defense against viruses

- Vaccination mobilizes the host immune system to prevent virus infections
  
  - Takes advantage of the memory system in the adaptive immune response

- **Key concept:** Vaccination breaks the transmission cycle of host-host spread in a population
Vaccines stimulate a protective immune response

They mimic an infection and provide memory

Initial immune response

Protective immunity

Immunological memory

Antibody and effector T cells

Time (days)

First infection

Mild or inapparent reinfection

(years)
Active vaccines stimulate immune memory

- 1781: outbreak of measles on Faroe Islands
- Next 65 years, islands free of measles
- 1846: another outbreak of measles; none of those who 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
Immune memory

- T and B lymphocytes that remain after infection has waned
- Maintain heightened ability to proliferate after infection
- Vaccines establish immune memory without pathogenic events typical of first encounter with virulent virus
Vaccines are now an integral part of our existence

• We immunize children, adults of all ages, as well as our domesticated and wild animals

• Because of immunization, many childhood diseases are rare
  - _Effects radical changes in society, populations, etc._

• Vaccines are a major part of the First World’s public health measures, **but not the Third World**
• Jenner, 1796
• Pasteur, 1885 - rabies vaccine; introduced the term vaccination from *vacca* (Latin, cow) in honor of Jenner
• Yellow fever, influenza vaccines - 1930s
Large-scale vaccination campaigns can be successful
Two simple, but fundamental ideas about how vaccines work and do not work in the real world

1. Herd immunity
   - Immunize ‘enough people’ to block virus spread
   - Not everyone has to be immune to protect the population

2. Maintenance of a critical level of immunity
   - If immunity of a population falls below a critical level, epidemics can easily occur even with an effective vaccine
   - Enough people have to be immune to block transmission in a population
   - Varies for virus and population
Population Immunity

- Virus spread stops when the probability of infection drops below a critical threshold
- The threshold is virus and population specific
- Smallpox: 80 - 85%
- Measles: 93 - 95%
- No vaccine is 100% effective
- When 80% of population is immunized with measles, 76% of population is immune
Public complacency is dangerous to any vaccine program

- “Viral diseases are a thing of the past”
- “Polio is long gone”
- “I never get the flu”
- “Measles is just a trivial kid’s disease”
- “Chicken pox only affects kids”
- “Kids should get infected naturally”
- “I’m not injecting anything into my body”
- “Vaccines make you sick, they cause autism, they cause multiple sclerosis, etc etc”
- “I know a guy who got the flu shot and then got the flu”
- “I can’t afford to immunize my kids”
- “I don’t have time this year”

Sound familiar?

When these attitudes prevail, society has serious problems with large-scale vaccination programs

*The programs are almost guaranteed to have mixed results at best*
Public healthy policy depends on complicated variables

- Poverty, social structure, infrastructure, and politics
  - All affect public health in the richest country in the world
- In developing countries, vaccines often are simply out of the question
- Large-scale immunization programs cost $$$$$, require government action
  - Not popular in a climate of cost-cutting
  - Not popular for those who oppose large social programs

Private money makes a difference
The Bill and Melinda Gates Foundation has taken a major lead in donating large sums of money toward world vaccination programs and research

Provides the financial resources to provide HBV vaccine to children in the poorest 74 countries of the world, and purchase of poliovirus vaccine for the eradication effort
• Public health costs money, not only for reagents, but also for the infrastructure to carry the measures
  - The dividing line between those countries that can afford vaccines is an annual gross national product of about $500 per person
  - Most of these ‘have-not’ countries are in sub-Saharan Africa

• Many countries do not want ‘Western ways’ imposed on them

• Technology and science can run afoul of personal preferences and beliefs
  - e.g. use of human fetal cells to grow viruses for vaccine production bothers many people who have strong views about abortion

In 1994, an English public schoolmaster banned the use of a measles/rubella vaccine because the vaccine used "the products of abortion" in its manufacture
How do you make a vaccine?

• Successful vaccination depends upon the induction of an *appropriate immune response*
  - *Remember the Th1 and Th2 response?*

• Gold standard: A vaccinated individual must be *protected against disease* caused by a virulent form of the specific pathogen
  - *Just getting ‘a response’ is not enough (e.g. producing antibodies)*
Vaccines can be *active* or *passive*

- Active - instilling into the recipient a modified form of the pathogen or material derived from it that induces immunity to disease
  - Generally useful for long term protection

- Passive - instilling the products of the immune response (antibodies or immune cells) into the recipient
  - Only useful for short term protection
  - Rabies immune globulin (RIG)
Rabies Immune Globulin (Intramuscular Route)

US Brand Names
- Bayrab
- HyperRAB S/D
- Imogam Rabies-HT

Description
Rabies immune globulin is used along with rabies vaccine to prevent infection caused by the rabies virus. Rabies immune globulin works by giving your body the antibodies it needs to protect it against the rabies virus. This is called passive protection. This passive protection lasts long enough to protect your body until your body can produce its own antibodies against the rabies virus.

Rabies immune globulin is given to persons who have been exposed (for example, by a bite, scratch, or lick) to an animal that is known, or thought, to have rabies. This is called post-exposure prophylaxis. Rabies immune globulin is used only in persons who have never before received the rabies vaccine.
A natural passive vaccine

Transient
low IgG levels

Months
Passively transferred maternal IgG

Years
IgM
IgG
IgA

Fraction of adult level
Conception 0 3 6 Birth 9/0 3 6 9 1 2 3 4 5 Adult
Serum immunoglobulin levels
Requirements of an effective vaccine

• Safety: must not cause disease, minimal side effects
• Must induce protective immunity in the population
  - Not every individual need 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)
<table>
<thead>
<tr>
<th>Disease or virus</th>
<th>Type of vaccine</th>
<th>Indications for use</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Live attenuated, oral</td>
<td>Military recruits</td>
<td>One dose</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>Inactivated whole virus</td>
<td>Travellers, other high-risk groups</td>
<td>0, 1, and 6 mo</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>Yeast-produced recombinant surface protein</td>
<td>Universal in children, exposure to blood, sexual promiscuity</td>
<td>0, 1, 6 and 12 mo</td>
</tr>
<tr>
<td>Influenza</td>
<td>Inactivated viral subunits</td>
<td>Elderly and other high-risk groups</td>
<td>Two-dose primary series, then one seasonal dose</td>
</tr>
<tr>
<td>Influenza</td>
<td>Live attenuated</td>
<td>Healthy children and adults 5–49 yr old</td>
<td>Two-dose primary series, then one seasonal dose</td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>Inactivated whole virus</td>
<td>Travelers to or inhabitants of high-risk areas in Asia</td>
<td>0, 7, and 30 days</td>
</tr>
<tr>
<td>Measles</td>
<td>Live attenuated</td>
<td>Universal vaccination of infants</td>
<td>12 mos of age; 2nd dose, 6 to 12 yr of age</td>
</tr>
<tr>
<td>Mumps</td>
<td>Live attenuated</td>
<td>Universal vaccination of infants</td>
<td>Same as measles, given as MMR</td>
</tr>
<tr>
<td>Papilloma (human)</td>
<td>Yeast- or SF9-produced virus-like particles</td>
<td>Females 9–26 yr old</td>
<td>Three doses</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Live reassortant</td>
<td>Healthy infants</td>
<td>2, 3, and 6 mo or 2 and 4 mo of age depending on vaccine</td>
</tr>
<tr>
<td>Rubella</td>
<td>Live attenuated</td>
<td>Universal vaccination of infants</td>
<td>Same as measles, given as MMR</td>
</tr>
<tr>
<td>Polio (inactivated)</td>
<td>Inactivated whole viruses of types 1, 2, and 3</td>
<td>Changing; commonly used for immunosuppressed where live vaccine cannot be used</td>
<td>2, 4, and 12–18 mo of age, then 4 to 6 yr old</td>
</tr>
<tr>
<td>Polio (live)</td>
<td>Live, attenuated, oral mixture of types 1, 2, and 3</td>
<td>Universal vaccination; no longer used in United States</td>
<td>2, 4, and 6–18 mo of age</td>
</tr>
<tr>
<td>Rabies</td>
<td>Inactivated whole virus</td>
<td>Exposure to rabies, actual or prospective</td>
<td>0, 3, 7, 14, and 28 days postexposure</td>
</tr>
<tr>
<td>Smallpox</td>
<td>Live vaccinia virus</td>
<td>Certain laboratory workers</td>
<td>One dose</td>
</tr>
<tr>
<td>Varicella</td>
<td>Live attenuated</td>
<td>Universal vaccination of infants</td>
<td>12 to 18 mo of age</td>
</tr>
<tr>
<td>Varicella-zoster</td>
<td>Live attenuated</td>
<td>Adults 60 yr old and older</td>
<td>One dose</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>Live attenuated</td>
<td>Travel to areas where infection is common</td>
<td>One dose every 10 yr</td>
</tr>
</tbody>
</table>
Inactivated vaccines

- Virions inactivated by chemical procedures (e.g. formalin, β-propriolactone, nonionic detergents)
- Infectivity is eliminated but antigenicity is not compromised
Poliomyelitis

Polio (grey), myelion (marrow) = Greek
itis (inflammation of) = Latin

“A common, acute viral disease characterized clinically by a brief febrile illness with sore throat, headache and vomiting, and often with stiffness of the neck and back. In many cases a lower neuron paralysis develops in the early days of illness”

Poliomyelitis
Inactivated poliovirus vaccine, IPV

- Poliovirus treated with formalin to destroy infectivity
- 1954: National Foundation for Infantile Paralysis-sponsored clinical trial of Jonas Salk’s IPV, 1,800,000 children. >50% protection, results announced 12 April 1955, licensed same day
SALK’S VACCINE WORKS!

Official Count

All Local Teams Are Rained Out

Only 3 Home Games

Salk’s Formulas

New York Times

Dr. Francis’ Official Report:

POLIO VACCINE IS ‘SAFE, EFFECTIVE AND POTENT’

Rain Washes Out Only 2 Shots

New York World-Telegram

Major Success Against Killing Paralytic Type

New York Post

Official Salk Test Report:

POLIO ROUTED!

55 Vaccine Will Be Even Better

New York Mirror

Triumph Over Polio!

Daily Mirror

They Vanished!

Polio Victims Fugitives

Only a Few Left
IPV

- Large stockpiles of vaccine released after licensure, within 10 days polio appeared in recipients
- 260 vaccine-associated cases in 94 immunized children, 166 contacts
- Due to incomplete inactivation of virus in lots produced by Cutter Laboratories
- 1955-1960 polio in US fell from 20,000 cases/yr to 2,500
THE Cutter Incident

HOW AMERICA’S FIRST POLIO VACCINE LED TO THE GROWING VACCINE CRISIS

Paul Offit, M.D.
Influenza

Three types: A, B, C
Inactivated influenza vaccine

• ~50,000 deaths/yr in US due to influenza virus

• Vaccine: virus grown in embryonated chicken eggs, formalin-inactivated or detergent or chemically disrupted virions

• A vaccine produced in cell culture has been approved by the EU, avoids egg allergies

• 75-100 million doses manufactured each year US

• 60-90% effective in healthy children and adults <65 yr

• Protection correlates with serum antibodies to HA, NA
Inactivated influenza vaccine

• Envelope proteins change each year; new strains must be selected in the first few months for manufacture
• Often use reassortants with most RNA segments from high-yielding strain, HA, NA from selected strain
• 2011-12 vaccine: A/California/7/2009 (H1N1); A/Perth/16/2009 (H3N2); B/Brisbane/60/2008
Subunit vaccines

- Break virus into components, immunize with purified components
- Clone appropriate viral gene, express in bacteria, yeast, insect cells, cell culture, purify protein
- Antigen usually a capsid or membrane protein
Subunit vaccine pro and con

• Advantages of a modern subunit vaccine
  - *Proteins produced by recombinant DNA technology*
  - *Contain no viral genomes that can replicate or escape*
  - *No contamination with infectious virus or foreign proteins*

• Disadvantages
  - *Expensive*
  - *Poor antigenicity (low level, short duration response)*
  - *Usually stimulate production of antibody, not cytotoxic T cells*
  - *Lack of good delivery system (injections are best, not well liked)*
Inactivated and subunit vaccines have a common problem

- Viral proteins don’t replicate or infect
- Don’t send out ‘danger signal’ to the immune response
- Example: failed respiratory syncytial virus
- Pure proteins often require *adjuvant* to mimic inflammatory effects of infection
Lack of antibody affinity maturation due to poor TLR stimulation leads to enhanced respiratory syncytial virus disease.
Adjuvants

- Substances that stimulate early processes in immune recognition, particularly elements of the inflammatory response

- Help produce a more robust acquired immune response with less antigen

- Work in at least three ways
  - Surface effects: through presentation of antigen as particles
  - Depot effects: localization of antigen to the site of inoculation
  - Inflammation effects: direct stimulation of the immune response

- In US vaccines
  - Aluminum salts in HBV vaccine; AS04 in Cervarix (aluminum hydroxide, monophosphoryl lipid A)
Some successful subunit vaccines

- Hepatitis B virus (HBV) - HBsAg protein produced in yeast
- Assembles into empty particles
Human papillomavirus vaccines

- Gardasil (Merck): types 6, 11, 16, 18 produced in *S. cerevisiae*
- Cervarix (GlaxoSmithKline): types 45, 31 produced in insect cells + AS04
- TWiV 126: Wart’s up, doc?
‘Live’, attenuated vaccines

- Viral replication occurs and stimulates an immune response
- Progeny virions may be contained to the site of replication
- Infection induces mild or inapparent disease
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 and may be a candidate for a vaccine.
Attenuation of poliovirus virulence

- Many researchers felt that IPV could not eliminate polio, and that a live vaccine that mimicked the natural infection would provide the most effective and durable immunity.

- 1952: Koprowski and Enders showed that passage of virus in cell culture could result in viruses with reduced virulence in animals.

- Albert Sabin’s three strains of OPV were licensed in the US in 1961.
Derivation of Sabin type 3 poliovirus vaccine

Type 3
P3/Leon/37
(isolate from fatal paralytic case)

21 passages in vivo (intracerebrally in monkeys)
8 passages in vitro (monkey testicle cultures)
39 passages in vitro (monkey kidney cultures)
3 plaque purifications (monkey kidney cultures)
3 passages in vitro (preparative, monkey kidney cultures)

P3/Leon 12a,b KP3/56 Sabin vaccine strain
<table>
<thead>
<tr>
<th>Virus</th>
<th>Mutation (location/nucleotide position)</th>
</tr>
</thead>
</table>
| P1/Sabin| 5'-UTR nt 480  
       | VP1 aa 1106  
       | VP1 aa 1134  
       | VP3 aa 3225  
       | VP4 aa 4065  |
| P2/Sabin| 5'-UTR nt 481  
       | VP1 aa 1143  |
| P3/Sabin| 5'-UTR nt 472  
       | VP3 aa 3091  |
OPV

Inactivated vaccine

Oral vaccine

Replication in oropharynx and intestine

Mucosal surfaces

Lymph node

Blood

IPV
Vaccine-associated poliomyelitis

- Recognized in 1950s that viruses excreted from recipients of OPV were more neurovirulent than vaccine
- First paralytic cases associated with type 3 reported in eastern Europe, early 1960s
- As indigenous polio was eliminated from the US (1979), only VAPP occurred, 7-8/yr, 1/1.4 million doses)
- US switched to IPV in 2000
Reported Cases of Paralytic Poliomyelitis, United States, 1961-2003

# Reversion of P3/Sabin

<table>
<thead>
<tr>
<th>Virus</th>
<th>Base at 472</th>
<th>Time of isolation after vaccination</th>
<th>Histological lesion score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabin vaccine</td>
<td>U</td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td>DM1</td>
<td>U</td>
<td>24 h</td>
<td>ND</td>
</tr>
<tr>
<td>DM2</td>
<td>U</td>
<td>31 h</td>
<td>1.58</td>
</tr>
<tr>
<td>DM3</td>
<td>U/C</td>
<td>35 h</td>
<td>ND</td>
</tr>
<tr>
<td>DM4</td>
<td>C</td>
<td>47 h</td>
<td>2.48</td>
</tr>
<tr>
<td>DM38</td>
<td>C</td>
<td>18 da</td>
<td>ND</td>
</tr>
<tr>
<td>P3/119</td>
<td>C</td>
<td>3-4 weeks</td>
<td>3.34</td>
</tr>
</tbody>
</table>

FluMist

- Live, attenuated, trivalent nasally administered influenza vaccine
- Each dose contains three current strains: H1N1, H3N2, B
- Reassortants of a master donor strain developed by serial passage at lower temperatures in primary chick kidney cells - HA, NA genes from current strains
- Viruses are cold-adapted, temperature-sensitive, and attenuated in a ferret model
- Replicate only in nasopharynx, produce protective immunity
Flumist - strain isolation

Wild-type Parental Virus
HA + NA Gene Donor
A/H1N1, A/H3N2, or B Virus

ca, ts, att Master Donor Virus
(PB2, PB1, PA, NP, M, NS)
A/Ann Arbor/6/60 (MDV-A) or
B/Ann Arbor/1/66 (MDV-B)

Co-infection

Selection for
ca, ts, att 6:2 Reassortant
Clonal isolation by limiting
dilution

1X egg pass

Master Virus Seed
(MVS)

1X egg pass

Manufacturer’s Working Virus Seed
(MWVS)

1X egg pass

Virus Harvest
(YH)

Trivalent Blend
A/H1N1 + A/H3N2 + B

CAIV-T Process

Purification

Monovalent
Bulk (MB)
Future influenza vaccines?

- Virus-like particles: synthesis of HA alone in cells leads to production of immunogenic particles
- Has also been done in plants
- 1 square meter of plants produces 20,000 doses at under $0.20/dose
Can viral diseases be eradicated?

• Smallpox eradication program was launched in 1967; disease declared eradicated in 1978
• Only major human disease to be eradicated
• For disease eradication, two features are essential
  - Replication must occur in only one host
  - Vaccination must induce lifelong immunity
• Two stocks of smallpox remain: CDC, Russia (?)
• virology.ws/2011/03/17/should-smallpox-virus-be-destroyed
Eradication of poliomyelitis

1988 WHA Resolution

2000 Stop poliovirus transmission

2005 Certify Global Eradication

2005-2010 Stop polio immunization
Polio eradication

- Polio vaccine differs from smallpox in that it can revert to a form that causes disease
- In countries using OPV, the only source of polio eventually is the vaccine
- Therefore, it has been decided that OPV use will cease after eradication has been achieved
- Assumptions for cessation of OPV: no non-human reservoirs of poliovirus, time-limited circulation of VDPV
### Wild Poliovirus - 2012

<table>
<thead>
<tr>
<th>Total cases</th>
<th>Year-to-date 2012</th>
<th>Year-to-date 2011</th>
<th>Total in 2011*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Globally</td>
<td>31</td>
<td>67</td>
<td>650</td>
</tr>
<tr>
<td>- in endemic countries</td>
<td>29</td>
<td>24</td>
<td>341</td>
</tr>
<tr>
<td>- in non-endemic countries</td>
<td>2</td>
<td>43</td>
<td>309</td>
</tr>
</tbody>
</table>

*Excludes viruses detected from environmental surveillance and vaccine derived polioviruses.
Problems associated with VDPV

- Recent outbreaks of polio in Egypt, Dominican Republic/Haiti, Philippines, Madagascar, caused by VDPV
- These VDPV strains regained virulence and spread in human populations
- Long-term persistence and excretion of VDPVs in immunocompromised persons
OPV transmission

• The recent polio outbreaks demonstrate that neurovirulent revertants of OPV can circulate for years (even in immune populations) and cause poliomyelitis

• These outbreaks mimic the situation that will occur when OPV usage is halted: circulation of neurovirulent revertants when vaccination coverage drops

• In light of this information, we cannot stop vaccinating
Vaccination against the vaccine

• After eradication, immunize globally with IPV
  - Higher cost than OPV
  - Not effective in tropical, underdeveloped countries

• Careful monitoring of environment for poliovirus
Why poliovaccine must be stockpiled after immunization ceases

- Virus in research laboratories
- Stored clinical and environmental samples
- Bioterrorism: synthesis of infectious DNA readily done
- Elimination of all sources of poliovirus is impossible; assume an outbreak will occur
Even if we eradicate a virus from the earth, as long as the nucleotide sequence is known...