Vaccines

Lecture 19
Biology W3310/4310
Virology
Spring 2014

Nothing shocks me. I’m a scientist.
INDIANA JONES
We survive the continuous barrage of viruses because our natural defense systems have the capacity to recognize and defend against ever-changing pathogens.
Vaccines are our proven best defense against viruses

- Vaccination mobilizes the host immune system to prevent virus infections
  - Immune memory

- Vaccination breaks the chain of transmission
• Jenner, 1796

• Pasteur, 1885 - rabies vaccine; introduced the term vaccination from *vacca* (Latin, cow) in honor of Jenner

• Yellow fever, influenza vaccines - 1930s
The Cow Pock — or — the Wonderful Effects of the New Inoculation!

Nith... the Publications of the Anti-Vaccinum Society.
Large-scale vaccination campaigns can be successful.
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

• Vaccines are a major part of the First World’s public health measures, but not the Third World
How vaccines work in the real world

- Maintenance of a critical level of immunity
- Herd immunity
Herd 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”

When these attitudes prevail, society has serious problems with large-scale vaccination programs
How do you make a vaccine?

• Induction of an *appropriate immune response*
  - *Remember the Th1 and Th2 response?*

• 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
  - *Long term protection*

- **Passive** - instilling the products of the immune response (antibodies or immune cells) into the recipient
  - *Short term protection*
A natural passive vaccine

Transient low IgG levels

Passively transferred maternal IgG

Serum immunoglobulin levels

Conception Birth Adult

Fraction of adult level

Months Years

0 3 6 9/0 3 6 9 1 2 3 4 5

IgM IgG IgA

Principles of Virology, ASM Press
Requirements of an effective vaccine

- Safety: no disease, minimal side effects
- Induce protective immunity in the population
- 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

- Chemical procedures (e.g. formalin, β-propiolactone, nonionic detergents)
- Infectivity is eliminated, antigenicity not compromised
Poliomyelitis

Polio (grey), myelon (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

POLIO VACCINE IS 'SAFE, EFFECTIVE AND POTENT'

POLIO ROUTED!

55 VACCINE WILL BE EVEN BETTER

Dr. Salk Visions 100% Effective
The Cutter Incident

- 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

M2 (ion channel)

M1 (matrix protein)

Lipid bilayer

HA (hemagglutinin)

NA (neuraminidase)

8 RNPs

(-) strand RNA

RNA polymerase

NP (nucleocapsid protein)
Inactivated influenza vaccine

- 3000-49000 deaths/yr in US due to influenza virus
- Vaccine: virus grown in embryonated chicken eggs, formalin-inactivated or detergent or chemically disrupted virions
- 75-100 million doses manufactured each year US
- 60% effective in healthy children and adults <65 yr
- Protection correlates with serum antibodies to HA, NA
- Vaccine produced in cell culture avoids egg allergies (Flucelvax)
Inactivated influenza vaccine

- Envelope proteins change each year; new strains must be selected in the first few months for manufacture
- Use reassortants with most RNA segments from high-yielding strain, HA, NA from selected strain
- 2013-14 vaccine: A/California/7/2009 (H1N1); A/Victoria/361/2011 (H3N2); B/Mass/2/2012; B/Brisbane/60/2008
Antigenic drift: Influenza virus
Subunit vaccines

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

Baculovirus Expression Vector System (BEVS) Technology

- Baculovirus
  - Engineer to express recombinant protein under powerful promoter

- expresSF+® cells
  - Infect cells in fermenter
  - Low-cost serum-free media

- Pure protein
  - Purify protein
  - Formulate with PBS into vaccine

Vaccine prepared within 2 months

Approved for 18-49 years old
Subunit vaccine pro and con

- Advantages of a modern subunit vaccine
  - Recombinant DNA technology
  - No viral genomes or infectious virus

- Disadvantages
  - Expensive
  - Poor antigenicity
  - Injected
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 16, 18 produced in insect cells
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
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
- Pure proteins often require *adjuvant* to mimic inflammatory effects of infection
Adjuvants

- Stimulate early processes in immune recognition
- Produce a more robust acquired immune response with *less antigen*
  - Slow release of antigen as site of inoculation
  - Inflammation
- Licensed
  - Alum (aluminum hydroxide or phosphate; in HBV vaccine) - US
  - AS04 in Cervarix (alum, monophosphoryl lipid A, TLR4 ligand) - US
  - MF59 - squalene oil-in-water emulsion (depot, innate stimulatory) - Europe

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Formula: \[ \text{Chemical Structure} \]
New vaccine technologies

Microneedle patch

Thermostabilization in silk (or sugars)
‘Live’, attenuated vaccines

- Viral replication occurs, stimulates immune response
- 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
FluMist

- Intranasally administered influenza vaccine
- H1N1, H3N2, B
- Reassortants of master donor strain developed by 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
Attenuation of poliovirus neurovirulence

Albert Sabin’s three strains of OPV licensed in the US in 1961
<table>
<thead>
<tr>
<th>Virus</th>
<th>Mutation (location/nucleotide position)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1/Sabin</td>
<td>5'-UTR nt 480</td>
</tr>
<tr>
<td></td>
<td>VPI aa 1106</td>
</tr>
<tr>
<td></td>
<td>VPI aa 1134</td>
</tr>
<tr>
<td></td>
<td>VP3 aa 3225</td>
</tr>
<tr>
<td></td>
<td>VP4 aa 4065</td>
</tr>
<tr>
<td>P2/Sabin</td>
<td>5'-UTR nt 481</td>
</tr>
<tr>
<td></td>
<td>VPI aa 1143</td>
</tr>
<tr>
<td>P3/Sabin</td>
<td>5'-UTR nt 472</td>
</tr>
<tr>
<td></td>
<td>VP3 aa 3091</td>
</tr>
</tbody>
</table>
Reported Cases of Paralytic Poliomyelitis, United States, 1961-2003


switch to IPV
# 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>

Eradication of poliomyelitis

1988    WHA Resolution

2000    Stop poliovirus transmission

2005    Certify Global Eradication

2005-2010    Stop polio immunization
Can viral diseases be eradicated?

- Smallpox eradication program launched 1967, eradicated 1978

- Two features essential for eradication:
  - Replication in only one host
  - Vaccination induces lifelong immunity
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
- It was decided to stop OPV after eradication
- Assumptions for cessation of OPV: no non-human reservoirs of poliovirus, time-limited circulation of VDPV
Problems associated with VDPV

• Outbreaks of polio in Egypt, Dominican Republic/Haiti, Philippines, Madagascar, caused by VDPV

• VDPV strains regained virulence, spread in human populations

• Long-term persistence and excretion of VDPVs in immunocompromised persons
OPV transmission

- Neurovirulent revertants of OPV can circulate for years (even in immune populations) and cause poliomyelitis
- VDPV outbreaks mimic the situation that will occur when OPV usage is halted
- 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
Wild Poliovirus - 2013
01 January - 29 October

Cameroon, Somalia, Kenya, Uganda

<table>
<thead>
<tr>
<th>Total cases</th>
<th>Year-to-date 2014</th>
<th>Year-to-date 2013</th>
<th>Total in 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Globally</td>
<td>51</td>
<td>16</td>
<td>407</td>
</tr>
<tr>
<td>- in endemic countries</td>
<td>44</td>
<td>16</td>
<td>160</td>
</tr>
<tr>
<td>- in non-endemic countries</td>
<td>7</td>
<td>0</td>
<td>247</td>
</tr>
</tbody>
</table>
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 outbreak will occur
- Which vaccine? OPV, IPV, new?
Virus Attenuation by Genome-Scale Changes in Codon Pair Bias

J. Robert Coleman,¹ Dimitris Papamichail,²* Steven Skiena,² Bruce Futcher,¹ Eckard Wimmer,¹† Steffen Mueller¹

Viral (+) strand genome

5' VPG UTR

OH AA(A)₉₀A OH₃'

UTR

Translation/processing

Capsid

Proteases and RNA synthesis

P1 P2 P3

VP0 VP3 VP1 2A 2B 2C P3

VP4 VP2

3AB 3CDpro

3AB 3B 3Cpro 3Dpol

Science June 27 2008
Even if we eradicate a virus from the earth, as long as the nucleotide sequence is known...