Nothing shocks me. I’m a scientist.

INDIANA JONES
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

**Longer life.** Anti-infection medicine and other factors have helped dramatically lengthen the average life expectancy in the U.S.
Vaccines stimulate a protective immune response

- **Initial immune response**
  - Time: 7 days
  - Antibody and T cell number increase

- **Protective immunity**
  - Time: 21 days
  - Antibody and T cell number remain elevated

- **Immunological memory**
  - Time: 2 years
  - Mild or inapparent reinfection

First infection

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• 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
Vaccines are now an integral part of our existence

- We immunize children, adults of all ages, 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 (e.g. rubella, measles)
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
Vaccine programs depend on public acceptance of their value

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Principles of Virology, ASM Press
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room number: virus

Herd immunity:

A. Demonstrates the importance of immunizing livestock
B. Emphasizes that not everyone must be immune to protect a population
C. Emphasizes that everyone must be immune to protect a population
D. Describes how group-think can dominate anti-vaccine choices
E. All of the above
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

**Diagram Description:**
- The x-axis represents time from conception to adulthood, with milestones including birth and years.
- The y-axis represents serum immunoglobulin levels as a fraction of adult values.
- At birth, there is a peak in IgG levels, followed by a decline and eventual stabilization.
- IgM levels increase over years, reaching adult values.
- IgA levels increase gradually, also reaching adult values over time.
- There is a mention of passively transferred maternal IgG, indicating the transfer from mother to baby at birth.
Zmapp, the best known passive vaccine

- Raised in mice immunized with virus-like particles
- Chimerized into human IgG1 scaffold
- Produced in tobacco plants
Passive therapy with convalescent serum

- Jordi Casals infected himself with Lassa virus at Yale in 1969
- Transfused with blood from nurse (Penny Pinneo) who had survived Lassa fever
Requirements of an effective vaccine

- Induction of an *appropriate immune response*
  - Th1 vs 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)
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)
## Viral vaccines licensed in the US

<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>Attenuated, oral</td>
<td>Military recruits</td>
<td>One dose</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>Inactivated whole virus</td>
<td>Travelers, 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>One dose seasonally</td>
</tr>
<tr>
<td></td>
<td>Recombinant proteins</td>
<td>Elderly; those with egg allergies</td>
<td>One dose seasonally</td>
</tr>
<tr>
<td>Influenza</td>
<td>Attenuated</td>
<td>Children 2–8 yr old, not previously vaccinated with influenza vaccine</td>
<td>Two doses at least 1 mo apart</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children 2–8 yr old, previously vaccinated with influenza vaccine</td>
<td>One dose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children, adolescents, and adults 9–49 yr old (e.g., FluMist, FluBlok)</td>
<td>One 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>Attenuated</td>
<td>Universal vaccination of infants</td>
<td>12 mo of age; 2nd dose, 6 to 12 yr of age</td>
</tr>
<tr>
<td>Mumps</td>
<td>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></td>
<td></td>
<td>Males 11–21 yr old</td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td>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>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 of age</td>
</tr>
<tr>
<td>Polio (attenuated)</td>
<td>Inactivated, 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>Variola virus</td>
<td>Certain laboratory workers</td>
<td>One dose</td>
</tr>
<tr>
<td>Varicella</td>
<td>Attenuated</td>
<td>Universal vaccination of infants</td>
<td>12 to 18 mo of age</td>
</tr>
<tr>
<td>Varicella-zoster</td>
<td>Attenuated</td>
<td>Adults 60 yr old and older</td>
<td>One dose</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>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
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SALK'S VACCINE WORKS!

POLIO VACCINE IS ‘SAFE, EFFECTIVE AND POTENT’

Cutter Incident

How America's first polio vaccine led to the growing vaccine crisis
Paul Offit, M.D.
Influenza virus

Three types: A, B, C

M2 (ion channel)
M1 (matrix protein)
Lipid bilayer

HA (hemagglutinin)
NA (neuraminidase)
NP (nucleocapsid protein)
RNA polymerase
8 RNPs
(-) strand RNA
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
- 2017-18 vaccine: A/Michigan/45/2015 (H1N1)pdm09-like virus; A/Hong Kong/4801/2014 (H3N2)-like virus; B/Brisbane/60/2008-like virus
Selecting an influenza virus vaccine

WHO GISM*
WHO CC†
WHO CC-CDC‡/FDA§
FDA
FDA
FDA
Manufacturers
Clinic

Surveillance
Select strains
Prepare reassortants
Standardize antigen
Assign potency
Review/license
Formulate/test/package
Vaccinate

*World Health Organization Global Influenza Surveillance Network
†WHO Collaborating Centres
‡US Centers for Disease Control and Prevention
§US Food and Drug Administration

http://www.microbe.tv/twiv/twiv-413/ on how strains are selected
Antigenic drift: Influenza virus
Go to:

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room number: virus

Which statement about inactivated viral vaccines is incorrect:

A. Chemicals can be used to inactivate infectivity
B. They do not replicate
C. They can be dangerous if inactivation is not complete
D. Antigenic variation can make them ineffective
E. None of the above are incorrect
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

expressSF+™ 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
Some successful subunit vaccines

*Cancer vaccine*

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

- Agents of warts (>170 types)
- Some are transmitted sexually, most common STD in USA
- Some cause low risk genital warts
- Others are high risk for cancers: cervix, vagina, penis, anus, oropharynx (31,000/yr; mostly 16, 18)
- Nearly half of Americans infected with genital HPV (18-59)

https://nyti.ms/2oFBTM2
**Human papillomavirus vaccines**

*Cancer vaccines*

- **Gardasil** (Merck): types 6, 11, 16, 18 produced in *S. cerevisiae*
- **Gardasil-9** (Merck): types 6, 11, 16, 18, 31, 33, 45, 52, 58
- **Cervarix** (GlaxoSmithKline): types 16, 18 produced in insect cells
- Should be given before becoming sexually active
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
Subunit vaccine pro and con

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

- Disadvantages
  - Expensive
  - Injected
  - Poor antigenicity
Inactivated and subunit vaccines have a common problem

- Viral proteins don’t replicate or infect
- Don’t cause inflammation
- 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
New vaccine technologies

Microneedle patch

Thermostabilization in silk (or sugars)
Universal influenza vaccine

- Broadly neutralizing human mAbs
- Prime-boost
- HA stem antigen

HK68 HA
HK68 Headless HA
Go to:

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room number: virus

**What are some requirements for an effective vaccine?**

A. Low cost  
B. Ease of administration  
C. Provides long lasting immunity  
D. Minimal side effects  
E. All of the above
Replication competent, attenuated vaccines

- Viral replication occurs, stimulates immune response
- Infection induces mild or inapparent disease
Empirically derived attenuated vaccines

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

- Replication competent, intranasally administered influenza vaccine
- Multivalent
- Reassortants of master donor strain - 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
Sabin oral poliovirus vaccine (OPV)
Attenuation of poliovirus neurovirulence

Albert Sabin’s three strains of OPV licensed in the US in 1961
## Determinants of Sabin vaccine strain attenuation

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1/Sabin</td>
<td>5’-UTR nt 480</td>
</tr>
<tr>
<td></td>
<td>VP1 aa 1106</td>
</tr>
<tr>
<td></td>
<td>VP1 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>VP1 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>
# 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>

Reported Cases of Paralytic Poliomyelitis, United States, 1961-2003

1 paralytic case/1.4 million doses
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
Poliovirus, 1988

Poliovirus, 1998

Poliovirus, 2008
(wild type 2 declared eradicated, no type 3 since 2012)
Even if we eradicate a virus from the earth, as long as the nucleotide sequence is known...
Engineering attenuated vaccines

- Yellow fever: first human virus identified, 1901
- Mosquito transmitted flavivirus
- Disease: fever and nausea to failure of major organ systems; high fatality
- Yellow fever vaccine 17D produced 1938 by 176 passages of virulent wild type Asibi strain in chick embryo tissue
- 500 million doses distributed; safe, effective
Building on success of YF 17D vaccine

Replace with dengue virus
Dengvaxia

- E, prM of dengue virus 1, 2, 3, 4 in YF 17D backbone
- Licensed in Mexico, Brazil, Philippines
- No protection against DENV-2
- Lead to worse disease in 2-9 yo
TV003

- Tetravalent, attenuated dengue virus vaccine produced by mutagenesis of infectious clone
- One dose, 100% protection vs challenge
Zika virus

- Membrane protein (M)
- Envelope (E) dimer
- RNA
- Capsid (C)

**Earliest evidence of Zika circulation**
- All types: <1960, 1960s, 1970s, 1980s, 1990s, 2000s, 2010s

- **Virologic**
  - Human
  - Mosquito
Zika virus

- Disease: rash, fever, joint pain, conjunctivitis, headache (similar to dengue, chikungunya)
- Incubation period 2-10 days
- 1 in 5 develop symptoms; 5 day course
- Fatalities rare
Central nervous system complications associated with Zika virus infection

<table>
<thead>
<tr>
<th>Adults</th>
<th>Infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Guillain-Barré Syndrome (post-infection autoimmune neuropathy; weakness, paralysis, death)</td>
<td>• Microcephaly</td>
</tr>
<tr>
<td>• Acute myelitis</td>
<td>• Lissencephaly</td>
</tr>
<tr>
<td>• Encephalopathy</td>
<td>• Macular atrophy</td>
</tr>
<tr>
<td>• Meningoencephalitis</td>
<td></td>
</tr>
</tbody>
</table>
Zika virus vaccines

- Attenuation
  - Replication competent natural virus vaccine
- Inactivation
  - Inactivated virus vaccine
- Cloning
  - Bacterial cell
  - Expression
    - Protein
      - Virus-like particle vaccine
      - Subunit vaccine
  - Cloning
    - Bacterial cell
    - Cloning
      - Replication competent virus vector vaccine
- Cloning
  - Human APC
  - DNA vaccine
Zika virus DNA vaccine
Vesicular stomatitis virus vector

Principles of Virology, ASM Press
For the US, a Zika virus vaccine will be a travel vaccine (like yellow fever vaccine and others)