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
Vaccines stimulate a protective immune response

- Initial immune response
- Protective immunity
- Immunological memory

Antibody prevalence and T cell number

Time (days)

First infection
Mild or inapparent reinfection

(2 years)
- 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

**Polio**
- Inactivated vaccine
- Oral vaccine replication-competent

**Measles**
- Vaccine

**Graphs:****
- Top graph showing reported cases of polio per 100,000 population from 1940 to 1990.
- Bottom graph showing reported cases of measles per 100,000 population from 1960 to 1990.
- Right graph showing estimated measles deaths (in millions) from 2000 to 2016.

Lives saved by measles vaccination

**Legend:**
- Estimated measles deaths in absence of vaccination
- Actual measles deaths with vaccination
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 western nations public health measures, **but not developing nations** (e.g. rubella, measles)
A key concept about how vaccines work: Herd Immunity

- Maintenance of a critical level of immunity
- Herd immunity = population scale immunity
Herd Immunity

- Virus spread stops when the probability of infection drops below a critical threshold
- The threshold is virus and population specific (e.g. $R_0$)
- Smallpox: 80 - 85%
- Measles: 93 - 95%
- No vaccine is 100% effective
- When 80% of population is immunized with measles, 76% of population is immune
$R_0$ for SARS-CoV-2 = 2-3

Number of people who must be vaccinated to prevent virus spread:

$$1 - \frac{1}{R_0}$$

Fraction of people who must be immune to prevent virus spread:

$$R_0 = \tau \cdot c \cdot d$$

$\tau$ = probability of infection given contact
$C$ = average duration of contact between infected and uninfected host
$D$ = duration of infectivity

50-70%
Vaccine hesitancy is dangerous to any vaccine program

- “Viral diseases are a thing of the past”
- “Herd immunity has not been proven to work”
- “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.
In some cases, medical exemptions to vaccination are indicated. These should not exceed 1% of the population, but they usually do as medical exemptions are inappropriately given.

**TWiV 496: Vaccines work, whether or not you believe in them**

https://www.microbe.tv/twiv/twiv-496/
Vaccine programs depend on public acceptance of their value
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

![Graph showing the development of IgM, IgG, and IgA antibodies over time from conception to adulthood.](image-url)
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
- Ongoing trials of convalescent plasma for COVID-19 patients
Passive therapy with mAb

- Mouse mAb chimerized into human IgG1 scaffold
- Human mAb isolated from B cells of patients

Zmapp

10.1126/science.abb2507
M. Yuan et al., Science 10.1126/science.abb7269 (2020)
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)
Replication competent attenuated virus vaccine

Inactivated virus vaccine

Nonrecombinant, purified subunit vaccine

DNA vaccine
mRNA vaccine

Replication competent virus vector vaccine

Human APC

Protein

Virus-like particle vaccine

Subunit vaccine
## 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></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Children, adolescents, and adults 9–49 yr old (e.g., FluMist, FluBio)</td>
<td></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 Males 11-21 yr old</td>
<td>Three doses</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>Reassortant</td>
<td>Healthy infants</td>
<td></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>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>Vaccinia 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>

### Ervebo - Ebolavirus vaccine

COVID vaccines authorized, not licensed

Pfizer, Moderna mRNA

J&J Ad26 vector
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

Reported paralytic polio cases and deaths in the United States since 1910

The reported figures include both wild- and vaccine-derived type polio infections that occurred indigenously and as imported cases.

Source: Our World In Data based on US Public Health Service (1910-1951) and US Center for Disease Control (1960-2010)

OurWorldData.org/polio/ • CC BY
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
- Cutter incident
Influenza virus

Three types: A, B, C

- HA (hemagglutinin)
- NA (neuraminidase)
- M2 (ion channel)
- M1 (matrix protein)
- Lipid bilayer
- 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
- Vaccines produced in cell culture (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
- 2020-21 vaccine: A/Hawaii/70/2019 (H1N1)pdm09-like virus; A/Hong Kong/45/2019 A(H3N2)-like virus; B/Washington/02/2019 (Victoria lineage) virus; B/Phuket/3073/2013-like (Yamagata lineage) virus [quadrivalent]
Selecting an influenza virus vaccine

http://www.microbe.tv/twiv/twiv-413/ on how strains are selected
Antigenic drift: Influenza 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
Recombinant zoster vaccine - Shingrix

- Recombinant gE produced in mammalian (CHO cells, secreted)
- Adjuvant with AS01
- Injected

Varicella-zoster virus
HBV vaccine

A 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)
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

**Nicotiana benthamiana**

Introduction of HA gene (transient or transgenic) → Harvesting and purification of HA
Subunit vaccine pro and con

- Advantages of a modern subunit vaccine
  - Recombinant DNA technology: fast
  - 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, poor activation of adaptive responses
- 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
  - AS01 (Shingrix; monophosphoryl lipid A, TLR4 ligand and saponin, stimulates innate immunity)
  - AS04 in Cervarix (alum, monophosphoryl lipid A) - US
  - MF59 - squalene oil-in-water emulsion (depot, innate stimulatory) - Europe
New vaccine technologies

Microneedle patch

Thermostabilization of influenza vaccine in sugars

https://www.nature.com/articles/s41598-019-44020-w
Universal influenza vaccine

By exchanging the HA head domains, but retaining the same HA stalk domain, the antibody response can be redirected towards the otherwise immuno-subdominant stalk region.

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 cells in culture

The virus is used to infect monkey cells

The viral genome acquires many mutations that allow it to grow well in monkey cells

The virus no longer reproduces 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)

**Graph:**
- **Polio**
  - Inactivated vaccine
  - Oral vaccine

**Diagram:**
- Virus enters through Mucosal surfaces
- Lymph node
- Blood
- Brain

- OPV
- IPV
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

switch to IPV
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
Global polio\textsuperscript{1} situation, previous 12 months\textsuperscript{2}

\textsuperscript{1}Excludes viruses detected from environmental surveillance;
\textsuperscript{2}Onset of paralysis 09 Sep. 2019 – 08 Sep. 2020

\begin{itemize}
  \item \textbf{WPV1 cases (latest onset)}
  \begin{itemize}
    \item Afghanistan: 56 (06-Aug-20)
    \item Pakistan: 145 (17-Aug-20)
  \end{itemize}
  \item \textbf{cVDPV1 cases (latest onset)}
  \begin{itemize}
    \item Philippines: 2 (20-Oct-19)
    \item Malaysia: 4 (14-Jan-20)
    \item Yemen: 14 (05-Jun-20)
  \end{itemize}
  \item \textbf{cVDPV2 cases (latest onset)}
  \begin{itemize}
    \item Chad: 74 (02-Aug-20)
    \item Somalia: 3 (18-Jul-20)
    \item Afghanistan: 69 (17-Jul-20)
    \item Sudan: 21 (18-Aug-20)
    \item DRC: 92 (28-Jun-20)
    \item Cote d'Ivoire: 29 (20-Jun-20)
    \item Nigeria: 4 (18-Jun-20)
    \item Ethiopia: 25 (13-Jun-20)
    \item Benin: 9 (12-Jun-20)
    \item Pakistan: 69 (13-Jun-20)
    \item Guinea: 8 (26-May-20)
    \item Burkina Faso: 10 (10-May-20)
    \item Togo: 17 (03-May-20)
    \item Ghana: 28 (09-Mar-20)
    \item Cameroon: 4 (11-Apr-20)
    \item Niger: 4 (15-Mar-20)
    \item Angola: 95 (06-Feb-20)
    \item Mali: 1 (06-Feb-20)
    \item CAR: 8 (05-Feb-20)
    \item Philippines: 12 (15-Jan-20)
    \item Zambia: 1 (25-Nov-19)
  \end{itemize}
\end{itemize}
New non-revertible poliovirus strains
The safety and immunogenicity of two novel live attenuated monovalent (serotype 2) oral poliovirus vaccines in healthy adults: a double-blind, single-centre phase 1 study


Summary
Background Use of oral live-attenuated polio vaccines (OPV), and injected inactivated polio vaccines (IPV) has almost achieved global eradication of wild polio viruses. To address the goals of achieving and maintaining global eradication and minimising the risk of outbreaks of vaccine-derived polioviruses, we tested novel monovalent oral type-2 poliovirus (OPV2) vaccine candidates that are genetically more stable than existing OPVs, with a lower risk of reversion to neurovirulence. Our study represents the first in-human testing of these two novel OPV2 candidates. We aimed to evaluate the safety and immunogenicity of these vaccines, the presence and extent of faecal shedding, and the neurovirulence of shed virus.
Even if we eradicate a virus from the earth, as long as the nucleotide sequence is known, any virus can be recovered.
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

A

5' C
UTR

Translation/processing

prM E NS1 2A 2B NS3 4A 4B NS5

Replace with dengue virus

B

Yellow fever vaccine DNA

In vitro RNA synthesis

5' (+) strand RNA transcript

Transfection

Cultured cells
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
SARS-CoV-2 virus vaccines

251 vaccines in development, 61 in clinical testing, 11 in use

Vaccine Categories:
- Inactivated Virus
- Live Attenuated Virus
- Protein Subunit
- DNA-Based
- RNA-Based
- Replicating Viral Vector
- Non-Replicating Viral Vector
- Virus-Like Particle
- Other Vaccines

Phases:
- Phase One
- Phase Two
- Phase Three
- Regulatory Review
- Authorized

Data as of 3/24/21
SARS-CoV-2 spike protein

Most vaccines (except inactivated, attenuated, and AstraZeneca) encode a pre-fusion spike
Two prolines added to C-terminal S2
Moderna mRNA-1273
1. Recruitment of immune cells to the site of administration

2. Migration of LNPs and APC to the draining lymph node

3. LNP uptake and antigen expression in cells at the injection site and in draining lymph nodes
Thoughts on COVID-19 vaccines

- Assessed by prevention of COVID-19
- Focus on induction of neutralizing antibody levels
- Variants of concern have changes in antibody epitopes
- However most T cell epitopes are not changed
- Reason why vaccines still prevent hospitalization and death even where VOC are circulating
- Future vaccines should incorporate more T cell epitopes
Next time: Antivirals