Pox viruses & Developing Viral Vaccines

- Molluscum contagiosum

- The Story of Smallpox:
  From Epidemics to Eradication to
  New Recombinant Vaccines

- Viruses as Vectors
  for vaccine development

Molluscum Contagiosum

Caused by a pox virus. The rash looks like one or more small growths or wart-like bumps that are usually pink, white, or skin-colored. These bumps often are soft and smooth and may have an indented center. They are commonly found on head, neck, trunk, upper arms, legs, and genital area. The rash will resolve on its own in 6-12 months with no treatment.
**Smallpox virus**

*Important Aspects*

- Double-stranded DNA, brick-shaped virus
- Causative agent of smallpox epidemics
- Vaccination developed from efforts to prevent smallpox

**Spread of Smallpox within the body**

- Infection of URT by inhalation of virus particles
- Incubation period
  - Days: 0-6
- Viral replication in the URT
- Viral replication in the lymphatics
- Primary viremia
- Viral replication in spleen, liver, etc
- Hemorrhage of small vessels of dermis leading to rash and "pox"
- Contagious period
  - Days: 12-18
Variolation: administering material collected from the lesions of smallpox victims that had been “inactivated” by the “physician”

Variolation was developed in China. Marco Polo bought the “technology” to Europe

Observations of a Country Doctor Led to the Development of a Vaccine for Smallpox

Observation of Dr. Edward Jenner in 1790s:
- During an epidemic of smallpox in the community, only the dairymaids were unaffected

Hypothesis:
- Contact with Cowpox lesions provides protection from smallpox

The ultimate experiment:
- Inoculate a child with scrapings from cowpox lesions
- Challenged child with infectious smallpox virus!
- Watch for signs of disease!!
Jenner’s experiment demonstrated the utility of a live, attenuated virus vaccine.

Vaccination was not universally well received.

Epilogue

We must not be enemies. Though passion may have strained it must not break our bonds of affection... when again touched, as surely they will be, by the better angels of our nature.

Abraham Lincoln
Crude Attempts to Improve the Coxpox vaccine resulted in Generating “Vaccinia”

- The coxpox scrapings were passed from community to community. Sometimes people would add various components (scrapings from other lesions, scraping from smallpox lesions) in an effort to improve the vaccine.

- When we were able to purify and characterize the vaccine, we found that a distinct virus had evolved. Vaccinia virus is related to, but distinct from either cowpox virus or smallpox virus.

Eradication of Smallpox

- Since humans are the only host, eradication is possible

- Strategy:
  - Identify cases
  - Quarantine victims
  - Vaccinate contacts and community
Properties of Smallpox That Led to Its Eradication

**Disease Characteristics**
- Smallpox disease always presents with visible pustular lesions
- Identification of sources of contagion allowed quarantine and vaccination of contacts

**Vaccine**
- Stable, inexpensive, easy to administer vaccine
- Presence of a scar indicates successful vaccination

Vaccinia Scar Indicating Successful Vaccination

![Vaccinia Scar Image]
Smallpox 1967

Smallpox 1972

Smallpox 1975-
WHO bounty system to find last cases
**Smallpox 1978**

- Last case of Smallpox was in 1978
- Smallpox virus stocks in USA and Russia
- Military recruits are vaccinated
- Worldwide vaccination ceased in the 1970s, estimated savings > $250 million/year in the USA

**Eradication of Smallpox**

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**Smallpox and Bioterrorism**

- Military recruits are vaccinated
- Should we go back to vaccinating everyone (to be prepared in case of a bioterrorism event)?
February, 2003
Public Health Nurse
Receives Smallpox vaccine
(policies discontinued in Jan 08 primarily due to vaccine related complications)

Complications associated with
Vaccinia virus vaccination
- Death: ~ 1 per million vaccinees
- Vaccine-induced diseases:
  - encephalitis
  - generalized vaccinia
  - eczema vaccinatum
These are more likely to occur in immunocompromised persons

Complications after Vaccination with Vaccinia Virus

Eczema Vaccinatum

This 27 year old man with a history of eczema was hospitalized 10 days after vaccination with vaccinia virus.

Story so far....

• success of the smallpox vaccine
• knowledge about low but detectable levels of complications
Can we exploit the knowledge gained through the use of vaccinia and use vaccinia-like viruses as inexpensive vaccine vectors?

**Vaccinia and Fowlpox**

*Promising Vaccine Vectors*

- Safety and efficacy established by smallpox vaccination programs
- Heat – stable
- Simple subcutaneous inoculation
- Can deliver many antigens in single dose

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**Replication of Pox Virus**

- Adsorption
- Penetration
- Loss of outer membrane
- Core release
- Early transcription
- Early mRNA
- Early enzymes
- Early virion proteins
- Uncoating of core
- Releasing viral DNA
- DNA Replication (2-5 hours)
- Early transcription
- Early mRNA
- Early enzymes
- Late virion proteins
- Late transcription
- Late mRNA
- Late enzymes
- Late virion proteins
- Morphogenesis (4-20 hours)
- Spherical immature particles
- Release primarily by cell disruption
Generating a Vaccinia Virus Vector for Rabies Virus

- Insert the gene encoding the Rabies virus glycoprotein into the Vaccinia virus DNA
- When animals are infected with the recombinant virus, the Rabies virus glycoprotein will be expressed, recognized as a foreign antigen and specific antibodies will be generated

Generating a Recombinant Vaccinia Virus

Problem: Endemic rabies in the fox population in Belgium
Large-scale vaccine “drop”

Vaccination with Recombinant Vaccinia Virus

Efficacy of Vaccine “Drop”

How about rabies control in the USA?
Elimination of arctic fox variant rabies in red foxes, metropolitan Toronto

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Human Vaccinia Infection After Contact with a Raccoon Rabies Vaccine Bait — Pennsylvania, 2009

Chicken pox

vaccina lesions

Hand, foot and mouth disease
### Poxvirus as a Vaccine Vector

- Inexpensive, easily administered vaccine
- Generates substantial immune response
- Used for rabies vaccination in wildlife
- Avian poxviruses, “Fowl pox” virus currently under study as a vaccine vector for humans

### Viruses as Vaccine Vectors

Many live-attenuated viruses can be modified to express proteins of interest

- recombinant Vesicular Stomatitis Virus (rVSV)
- Adenovirus – such as chimp Ad
- Yellow Fever 17D chimeric viruses

An Ebola deletion is also being evaluated as a live attenuated vaccine

### Ebola Vaccines

- rVSV-EBOVGP – multiple versions
- ChAd-EBOVGP
- EBOVdeltaVP30
- All protect nhp from challenge with Ebola virus

- Phase 1 in humans indicates minimal adverse events and induction of neutralizing Ab
Targeting Zika Virus

- Determine if any DAAs directed against the flavivirus HCV also inhibit Zika

- Evaluate killed virus, live-attenuated and chimeric virus vaccines
Can we make a vaccine to Zika virus?

Cryo-EM Structure of Zika Virus (Science, March 2016)

YFV 17D genome cloned as cDNA

5'   prM   E   Non-Structural genes   3'

Exchange with coat protein genes of DENV, JEV or WNV

5'    E   prM   Non-Structural genes   3'

3'  Chimeric cDNA transcribed to RNA

Virus grown in cell culture

Envelope is the immunizing Ag from a heterologous flavivirus

RNA replication machinery is from YFV 17D

DNA vaccine expressing ZV-prM-Env; rAdeno52-ZV-prM-Env

Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys
Smallpox was eradicated by an effective vaccine. Ceasing vaccination saves $\$$(cost of vaccination and cost of side effects).

Summary

Modifying poxviruses can be an effective way to generate vaccines against other pathogens. V-RG is a success story….

Modifying viruses to make vaccines for Ebola and Zika is in progress….

GOOD LUCK
ON
THE BOARDS!!