Viral Disease and Prevention
BioScience in the 21st Century
November 2018

Robert Buckheit III, Ph.D
ImQuest BioSciences, Inc.
Robert.Buckheit@imquestbio.com
Outline

• Case Study – Emergence of a New Virus
• What is a Virus?
  • History and discovery
  • Basic viral replication and transmission
• Influenza: How Do Viruses Change Over Time?
• Ebola: Zoonotic Transmission and Reservoirs
• HIV: Antiviral Drugs and Principle of Combinations
• Vaccination: Principles and Challenges
Mystery Illness -- 1981

- Adult Male walks into the Hospital
- Mid thirties, severe case of community acquired pneumocystis carinii (yeast)
- Pale, very skinny—evidence of other unusual infections
- 2 weeks prior the patients was healthy, no other underlying health factors
- Within 48 hours, patient dies of pneumonia
- Immune system is damaged (No CD4 T cells cells)
- Strange?—Maybe, but its just one patient
Other reports of CD4 Loss in Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Percent of PBMC*</th>
<th>CD4/CD8 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD4+</td>
<td>CD8+</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>57</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>47</td>
</tr>
<tr>
<td>Mean</td>
<td>3</td>
<td>53</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>46</td>
<td>28</td>
</tr>
</tbody>
</table>

*PBMC = peripheral blood mononuclear cells (lymphocytes and monocytes)
The disease spreads...1982-1983

- No one knows what is causing the illness
- Originally, only seemed to infection Homosexual males
- January 1982, the first clinic for patients with this disorder opened in San Francisco
- September 24th, the term AIDS is coined—**Acquired Immune Deficiency Syndrome**
- December 10th—AIDS described in young infants who received blood transfusions (blood borne illness?)
- First reported cases of women with a syndrome similar to AIDS
- CDC hosts a meeting to talk about the blood supply—criteria for testing, and no consensus is reached
- Also, reports of AIDS in Haitian populations—is AIDS going global?
- Many though this disease pattern to be consistent with a infectious agent...mortality rate of infected patients is almost 100%

Source: CDC HIV AIDS surveillance report 2003
Discovery of the Causative Agent

• In May 1983--First reports from a French Research group describing Lymphadenopathy Associated Virus (LAV)
  • Professor Luc Montagnier (won the Nobel Prize in 2008)
  • In November Dr. Robert Gallo’s lab in the USA also propagates LAV in immune cells (NIH)

• AIDS was caused by a newly discovered virus (renamed HIV)

• New virus jumped into the human population
So...What is a Virus?

Discovery, Structure and Spread
Definition

• “An infective agent that typical consists of a nucleic acid molecule (genome) inside a coat protein, is too small to be seen by light microscopy, and is able to multiple only within the living cells of a host”
Discovery of Viruses – First identified in plants

• **1892** – Dmitri Ivanovskiy, studied tobacco mosaic disease, and discovered that the disease causing agent was able to pass through a ceramic filter that trapped all bacteria
  - Thought the agent was a toxin

• **1898** – Martinus Beijerinck confirmed this discovery and terms the causative agent a “contagium vivum fluidum” or “soluble living germ” (not a toxin)
Discovery of Human Viruses

• **1897** – Friedrich Loeffler discovered a similar agent was responsible for foot-and-mouth disease
  • Viruses also infect humans
• **1901** – Walter Reed discovered that yellow fever was caused by a virus passes by mosquitos (panama canal)
  • Mosquito vector theory was first proposed by Carlos Finlay in 1881...verified by Walter Reed Commission (human testing)
Wide Variety of Shapes and Sizes

- Likely that there is a virus that infects every living organism on the planet – animals, plants even bacteria
- Typically comprised of a protein capsid (coat) surrounding nucleic acids (genome).
- Sometimes has a lipid envelope surrounding protein coat
How do viruses infect your cells?

• **Endocytosis (protein import):** How cells take in resources from the outside world
  • All cells do this at all times, this is how cells “drink” or take up resources

• Process is mediated by **receptors** on the surface of cells
  • **Receptors** on proteins attach to particle and the particle is ingested
  • **Very specific:** “Lock and key”

• **Viruses mimic this process to invade cells**
What does this look like...
Viruses use Mimicry to Infect Cells

• Many Viruses infect cells in the respiratory tract
• Example: Influenza Hemaglutinin (virus protein) binds to Salic Acid Receptor on respiratory tract cells
  • Virus enters the cell through endocytosis... viral replication occurs
  • Influenza replicates in these cells, makes copies of itself and causes symptoms of disease
  • Cell is now infected... virus can spread to other cells

• Virus protein and Receptor Interaction determines what cell type viruses can infect
  • And your symptoms
Wide variety of Illness

• Many make people sick and can result in death

• Viruses affect every organ and system in the body

• Why study viruses? – Many cell biology functions have been discovered by studying how viruses manipulate the human body

• Best viruses never cause disease to the host (and never kill the host)
Many Ways to Spread Viruses

• **Aerosol** – When you sneeze or cough, you make small, tiny water droplets or “aerosols”, many viruses and bacteria can fit in these droplets and can be inhaled
  • Influenza, RSV

• **Direct Contact** – bacteria or viruses transmitted by contact or by bodily fluids
  • Ebola (bodily fluids), HIV (bodily fluids)

• **Oral** – transmission by food or other particles entering the mouth
  • Cruise Ship Viruses (Norwalk Virus)—food poisoning
  • Fecal-Oral transmission—Rotavirus (stomach flu, and diarrhea in infants)

• **Fomite** – non-living objects such a bedding, towels toys and barbed wire or nails that can cause disease
  • Biological warfare, also Influenza and childhood diseases (sharing toys in daycare)

• **Zoonotic** – Transmission from animals to humans
  • Swine Flu, Ebola
Influenza

How Do Viruses Change Over Time?
Influenza Virus

• Infectious disease of birds and mammals
  • Causes mild to severe respiratory illness
  • Serious illness results in hospitalization or death
  • Children and older adults are at high risk for flu infection
  • Best defense is to get a flu vaccine every year
  • Infects cells that line the respiratory tract

• Viral genome composed of 8 pieces of RNA in side a protein coat
Flu Symptoms

Symptoms of Influenza

Central
- Headache

Systemic
- Fever
  (usually high)

Muscular
- (Extreme) tiredness

Joints
- Aches

Nasopharynx
- Runny or stuffy nose
- Sore throat
- Aches

Respiratory
- Coughing

Gastric
- Vomiting
Aerosol or Fomite Transmission of Influenza
Seasonal Influenza is typical and usual predictable
Recurring and Frequent Influenza Pandemics
Spanish Flu Pandemic 1918

• Killed between 20 and 40 million people—more then all of the soldiers who died in World War I

• Likely exacerbated by an increase in world travel after WWI

• Infected 28% of all Americans
Swine Flu Pandemic of 2009

H1N1 Swine Flu: Similar Influenza Symptoms in Pigs

Intestinal
- Diarrhea

Respiratory
- Coughing
- Sore throat

Physical
- Lethargy
- Lack of appetite

Nasopharynx
- Sneezing
- Mucous: nose/eye

Systemic
- Fever
- Weight loss
- Poor growth

Symptoms of Swine influenza
Influenza Infects Many Animals Species
Results in Influenza Mutations Over Time

**Mutation**

*Antigenic drift*

Small Mutations

Seasonal Influenza

**Antigenic shift**

Mixing Different Animal Strains

New Strain

Pandemic Influenza
H1N1 Swine Flu: Result of Antigenic Shift

- Pig (swine) was infected with an avian and human influenza virus at the same time.
- Viruses mixed, resulting in a virus with new viral proteins, that could infect humans.
- No human had ever seen the combination – no immunity in the population.
- Pandemic Influenza is caused by Antigenic Shift – typically much more lethal.
Many believe the next deadly pandemic will be a result of avian influenza jumping into the human population. Avian Influenza assisted the jump from Swine to humans in 2009.

Zoonotic Transmission:
- Disease that is transmitted between vertebrate animals and humans.
- Cross species jump into the human population.
  - Often, host species is not affected by virus or acts as a reservoir.
  - Unless this reservoir is eliminated, the viruses can still be transmitted to humans and cannot be eradicated.
Ebola
Zoonotic Transmission and Reservoirs
Discovery in 1977 and Current Distribution

Endemic to regions of Africa; also isolated in Philippines from Non-human primates

Figure and data: CDC
Transmission and Pathogenesis

- Transmission: Bodily fluids and ingestion of infected meat
- Requires direct contact with contaminated fluids
  - Virus has been detected in many body fluids, making transmission easier than for a virus like HIV
    - Skin (sweat)
    - Saliva
    - Urine
    - Feces
    - Breast milk
    - Semen
    - Vaginal fluid

[Diagram of Ebola virus' typical path through a human being]

© 2014 MCT
Source: U.S. Centers for Disease and Control, BBC
Graphic: Melina Yingling
West Africa outbreak of 2014-2015

- First outbreak in a highly populated area
- 28,600 cases in Guinea, Sierra Leone, and Liberia (11,325 deaths)
- Cases in Nigeria, Spain, US, Mali and Senegal
- Additionally, separate outbreak in Dem Rep of Congo
2018 Current Ebola Outbreak

• 44 Ebola Cases, 36 Deaths

• Outbreak are likely to continue in endemic areas

• Current vaccination of rVSV-ZEBOV is being tested in the field (unknown efficacy)
Ebola Treatment 37 years after discovery...not much has changed

1977

2014
Zoonotic Cycle and Reservoir...outbreaks likely to continue if animal reservoir is maintained
Also true for mosquito born viruses...zika/dengue/chikungunya/west nile
HIV

How Do we Prevent Viral Infection?
What characterizes AIDS?

- Immune system is broken...
- Once your CD4⁺ T cell count falls well below healthy levels, rise in opportunistic infections
- Infections that arise from pathogens that are normally harmless in healthy individuals
- Your Immune system is no longer functioning

- HIV never actually kills you---other viruses, bacteria and fungus do
- HIV kills your immune system and response to infections
Opportunistic Infections

- Common yeasts, part of normal flora
- Cause of thrush: superficial infection of the oral cavity
- Can be invasive in the GI tract especially in the esophagus making it hard to swallow (dysphagia)

- Non HIV related: Skin Cancer affecting elderly, Jewish men
  - Mainly lesion on the feet, benign
- HIV Pandemic: Severe, aggressive form of the disease
- KSHV or HHV-8 (Human Herpesvirus-8) identified as causative agent in 1994 (also a virus)
- Today, significant morbidity and mortality in subequatorial African countries
HIV: Discovery of Antiretroviral Drugs

• Earliest Drug was AZT (Zidovudine)
  • Originally a cancer therapeutic, was found to be active against HIV
  • At the original dose, was highly toxic to patients—but did work to control infection for a period of time
• Used alone, only extended life for about a year
  • virus mutated and AZT was no longer effective

• HIV mutates quickly resulting in resistant to antiviral drugs
HAART—Combination Antiviral Treatment

- **Highly Active AntiRetroviral Therapy**
  - Use three or more drugs at one time to stop viral replication

- Combination drugs are powerful and prevent HIV from mutating and becoming resistant to therapy

- Taken daily, makes HIV a “chronic condition”

Source: CDC HIV AIDS surveillance report 2003
Multiple Drugs Available for HIV Treatment and Prevention
More Limited Options for Influenza Treatment…but maybe this is changing…

---

More Evidence That Baloxavir Reduces Flu Symptom Duration
— Investigational antiviral matches or exceeds efficacy of oseltamivir

By Liz Highleyman, Contributing Writer, MedPage Today
October 08, 2018

Baloxavir Reduces Flu Symptoms and Viral Shedding

Diana Phillips
September 05, 2018

Genentech Announces FDA Approval of XOFLUZA (Baloxavir Marboxil) for Influenza
— First and only single-dose oral medicine approved to treat the flu —
— XOFLUZA significantly reduced the duration of flu symptoms compared to placebo —
— First novel proposed mechanism of action to treat the flu in nearly 20 years —

October 24, 2018 01:06 PM Eastern Daylight Time
Antiviral Drugs

• Lots of pharmaceutical and small biotech development activities for various viruses
  • Recent success in some: HIV, HCV (HIV latency still prevents total cure)
• Resistance and limited options in others: Influenza
  • Lack of combinations increases chance of resistance, new drugs still needed!
  • Great news that another antiviral for influenza is available
• Failure in current treatment for many: HBV, Ebola, Dengue/Zika

• Antivirals can only treat you once you are infected, so how can we prevent viral infection in the first place?
• How can we reduce animal reservoirs?
Virus Prevention: Vaccines

Gold Standard for the Prevention of Infection
Edward Jenner

• Known as the father of Modern Immunology
• Famous experiment of 1796
  • Smallpox is the scourge of the 18th century world
  • Folklore that milkmaids who suffered from cowpox never got small pox
    • Jenner hypothesized that exposure to cowpox prevented smallpox infection
  • Key experiment: exposed 8 year old James Phipps to pus from a cowpox pustule, and then exposed James to small pox (he survived, luckily)
• Vaccine provided *Protective Immunity*

• First “vaccine”
  • Vaccines were named in honor of Jenner
  • Vacca is cow in latin, hence Vaccine (in honor of the cow pox story)
There has always been backlash against vaccination...

1802: Satirical cartoon implying that those who got vaccinated turned into cows

2018: Nothing new, but still not helpful for human health...
Vaccination is incredible powerful and effective

- Smallpox has been eradicated from the globe

- Polio is undergoing an eradication campaign

- Measles, chickenpox and other childhood diseases are almost non-existent in the United States
  - Unless parents don’t vaccinate their children

![Graph showing the decrease in smallpox cases](image1)

![Graph showing the decrease in measles cases in the United States](image2)

**How one unvaccinated child sparked Minnesota measles outbreak**

**CDC reports biggest measles outbreak since 1996**

Anti-vaccine movement is giving diseases a 2nd life
Childhood Vaccinations: Global Success, vast reduction in childhood diseases

<table>
<thead>
<tr>
<th>Vaccine given</th>
<th>1 month</th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
<th>12 months</th>
<th>15 months</th>
<th>18 months</th>
<th>4–6 years</th>
<th>11–12 years</th>
<th>14–16 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria–tetanus–pertussis (DTP/DTaP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Inactivated polio vaccine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measles/mumps/rubella (MMR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumococcal conjugate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemophilus B conjugate (HiBC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varicella</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
How Vaccines Work: Herd Immunity

• Basic idea came from protecting groups of animals—hence “herd”

• Vaccination of a portion of the herd (less cost) and end up with full protection (great)

• Same principle works in human populations (“herds”)

![Diagram showing how herd immunity works.](image)
More work to be done...

<table>
<thead>
<tr>
<th>Disease</th>
<th>Estimated annual mortality</th>
<th>Estimated annual incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>1,086,000</td>
<td>300–500 million</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>14,000</td>
<td>no numbers available</td>
</tr>
<tr>
<td>Worm infestation</td>
<td>16,000</td>
<td>no numbers available</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1,498,000</td>
<td>~8 million</td>
</tr>
<tr>
<td>Diarrheal disease</td>
<td>2,213,000</td>
<td>~4,100 million</td>
</tr>
<tr>
<td>Respiratory disease</td>
<td>4,039,000</td>
<td>~362 million</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>2,673,000</td>
<td>~2 million</td>
</tr>
<tr>
<td>Measles</td>
<td>875,000</td>
<td>~44 million</td>
</tr>
</tbody>
</table>
Summary

• Viruses are ancient and infect all organisms on the planet
• All evolve over time as a result of small random mutations
• Mixing of different virus strains results in “brand new viruses”
• Constant battle of evolution of virus and host
• Viral therapy is most effective in combination...reduces chance of mutation

• Zoonotic transmission is a key factor is viral epidemics, pandemics, and maintenance of virus in the population
  • Must eliminate animal reservoir to eliminate jumps back into the human host
• Vaccines are the best protection against infection
  • Can protect you and others from infection (and animals)
Thanks! Any Questions?

DON’T GET THE FLU. DON’T SPREAD THE FLU.

GET VACCINATED.

cdc.gov/flu