Viruses, viroids, prions

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Microbiology
Virus: a brief history

• Used to refer to any poison
  - Later = causative agent of any disease
  - Pasteur = term for pathogenic bacteria

• Frederich Loeffler/Paul Frosch (1898)
  - Showed the foot & mouth disease caused by a “filterable agent”

• TMV
  - Dutch microbiologist, Martinus Beijerinck
  - Russian scientist who showed that this was a filterable agent = D. Ivanowsky
Filterable agents

- Increased number of filterable agents discovered = filterable viruses
- Bacteriophage discovered independently by F.W. Tort and F. d’Herelle (1917)
- What are they?
  - Are viruses alive?
  - Should the definition of life be revised to include viruses?
Characteristics

• Obligatory intracellular parasite
• Uses existing machinery of host cell for replication
• Causes synthesis of specialized structures to transfer nucleic acids to other cells
• All life forms are parasitized by specific virus
• Virus that is not in host cell = virion
  - Metabolically inert
  - No respiratory or biosynthetic function
General structure

- Single type of nucleic acid surrounded by protein capsid
- Sometimes lipid envelope with integral proteins and carbohydrates derived from host cell membrane
- Size range = 20-14,000 nm in length
Nucleic acid

• Can be either DNA or RNA, not both at same time
• May be double stranded, single stranded, circular or divided into separate segments
• 3 major divisions
  - DNA viruses
  - RNA viruses
  - Contain DNA and RNA at different times in cycle
    • Retroviruses = RNA genome, replicate through DNA intermediate
    • Hepatitis B virus = DNA in virion, RNA intermediate in replication
Viruses divided into 6 groups based on structure of genome and replication methods

- **Class 1:** viruses with dsDNA
  - dsDNA $\rightarrow$ mRNA $\rightarrow$ protein
- **Class 2:** viruses with ssDNA
  - ssDNA $\rightarrow$ dsDNA $\rightarrow$ mRNA $\rightarrow$ protein
- **Class 3:** viruses with dsRNA
  - dsRNA $\rightarrow$ mRNA $\rightarrow$ protein
- **Class 4:** viruses with ssRNA$^+$ (same polarity as mRNA)
  - mRNA $\rightarrow$ protein
- **Class 5:** viruses with ssRNA$^-$
  - ssRNA $\rightarrow$ mRNA $\rightarrow$ protein
- **Class 6:** viruses with ssRNA$^+$ and RT or RNA-dep DNA polymerase
  - ssRNA $\rightarrow$ ssDNA $\rightarrow$ dsDNA $\rightarrow$ mRNA $\rightarrow$ protein
Differences in requirements for viral replication

- DNA viruses = need genes to code for capsid proteins and/or spikes
- RNA viruses = need the same genes as DNA viruses plus genes for enzymes to allow viral RNA to be replicated
Capsids

• Four basic categories of viruses based on structure
  - Polyhedral (icosahedral)
  - Helical
  - Enveloped
  - complex

• Most capsids rod shaped or spherical
  - Usually one or two basic shapes to keep genome simple
  - Subunits self assemble with help of some chaperone protein
    • Aid in folding but not part of final structure
Capsid assembly

trisymmetron
20 per virus

pentasymmetron
12 per virus

surface reconstruction
density map

Capsomer
Although the majority of the capsomers appear hexagonal, they are trimers, composed of three protein subunits.
The central capsomer of each pentasymmetron is a pentamer.
Envelopes

• Combination of lipids, proteins, carbohydrates
• Formed by budding
• Proteins in envelope are virus specific
  - Host cell proteins are excluded
Envelopes

- Some contain additional structures, such as spikes
  - Composed of carbohydrate + protein
  - Project from surface
  - Used in attachment and ID
  - peplomeres
Viral taxonomy

• Very controversial
• Current basis of classification
  - Nucleic acid, morphological class, presence or absence of envelope
• Virus names
  - Family = -viridae, Genus = -virus, no species name
• Viral species
  - Group of viruses sharing same genetic information and ecological niche
• Descriptive common names for ID
Isolation and cultivation

- **Bacteriophage**
  - Can be grown in culture media = solid or liquid
  - **Solid media**
    - Grow an lawn and inoculate with virus
    - Look for plaques
  - **Liquid media**
    - Mix bacteriophage with bacteria and melted agar and pour into plate
    - Look for plaques
  - Each plaque = single virus in initial suspension
- Concentrations of viral suspensions measured by number of plaques = pfu’s
A typical set of serial dilutions of the bacteriophage T4.
Isolation and cultivation: Animal viruses

- **In animals**
  - Inoculate and look for disease or sacrifice and examine tissues
  - Animal models for specific viruses and diseases

- **In embryonated chicken eggs**
  - Undifferentiated tissues = many viruses will grow
  - Drill the eggs, inoculate near one of membranes
  - Death of embryo, or CPE in membranes
  - Used to attenuate viruses (subphysiological temperature)

- **In cell culture**
  - Lines grown to confluence
  - Inoculate with dilutions of viruses and look for CPE
  - Primary cell lines vs transformed cell lines
Figure 1. Measles cytopathic effect (CPE) in B95a cells. Top photo shows uninfected B95a.
Viral identification

• Visualization
  - Electron microscope

• Serology
  - Western Blot most common (viral reaction with antibody)

• RFLP
  - Restriction fragment length polymorphisms

• DNA fingerprinting

• PCR
Viral replication (animal virus)

- Attachment
- Penetration
- Uncoating
- Transcription
- Translation
- Replication
- Assembly
- Release
Viral replication

- **Attachment**
  - Virus binds to specific receptor on host cell
- **Penetration**
  - Plasma membrane invaginates = pinocytosis
- **Uncoating**
  - Viral capsid attacked by cellular enzymes
  - Nucleic acid released into cytoplasm or nucleus
- **Transcription**
  - In cytoplasm or nucleus
Viral replication

• Translation
  - Proteins made from viral-directed mRNA needed for synthesis of new viral particles

• Replication of viral nucleic acids
  - Use original viral nucleic acid as template
  - Directed by specialized enzymes coded by viral genome

• Assembly
  - Viral nucleic acids and capsid subunits are assembled

• Release
  - Lysis or budding
Lytic cycle of bacteriophage

- Attachment or adsorption
- Penetration
- Biosynthesis
- Maturation
- Release
Lytic cycle

• **Attachment**
  - Bacteriphage and bacteria collide
  - Virus attaches to complementary receptor

• **Penetration**
  - Viral enzyme breakdown cell wall
  - Nucleic acid injected into bacterium
  - Capsid remains on outside

• **Biosynthesis**
  - Synthesis of viral nucleic acids and proteins
  - Virus induces degradation of host DNA
  - Eclipse period = “factory” gearing up and parts made
Lytic cycle

• **Maturation**
  - Spontaneous assembly into complete virions

• **Release**
  - Lysis of host cell by production of lysozyme by phage gene
  - Release of virions

• **Burst time**
  - 20-40 minutes

• **Burst size**
  - 50-200 per cell
1. Start of infection. 
Virus DNA enters host cell. Protein coat does not.

2. Virus DNA directs the production of new virus particles.

3. End of infection. 
New generation of virus particles burst from host cell.
Lysogenic cycle (Bacteriophage λ)

- Lysogeny = incorporation into host DNA
  - Latent period in animal cells
- Trigger stimulates cell to enter lytic cycle
- 3 important results
  - 1. Lysogenic cells are immune to infection by same virus
  - 2. Host cell may exhibit new properties
    - *C. diphtheriae* requires presence of temperate virus to produce toxin associated with disease
  - 3. Possibility of specialized transduction
    - Packaging of host DNA along with viral DNA in capsid
Viruses and pathology

• Are there good viruses?

• Cancer
  - Linked with induction of certain cancers
  - Studies on chicken leukemia by Ellerman and Bang in 1908 showed that cancer can be induced by viruses
  - Another mechanism for altering genetic material in cell (others = radiation, chemical exposure, etc.)
Viral diseases

- HIV
- HSV
- Variola
- Varicella zoster
- HBV
- HPV
- Polio
- HDV
HIV

• Spiked, enveloped retrovirus
  - Glycoprotein spike gp120 attaches to CD4 receptor of T-cells, macrophage, some lymphocytic and epithelial cells

• Lysogenic cycle
  - viral RNA is converted to DNA and incorporated into host cell DNA
  - Not detected by immune system

• Retrovirus prone to errors during replication
  = new varieties constantly produced
  - Syncitial spread
HIV structure

gp120 (SU)
gp41 (TM)
p55 (RT), p63
p11 (IN)
p6, 9 (NC)
p24 (CA)
p17 (MA)
Three clinical stages

• No symptoms other than lymphadenopathy
• Decrease in T-cell count
  - Secondary infections such as *C. albicans*, shingles, etc.
• Full blown AIDS
  - T-cells <200/mm3
  - More infections including *C. albicans*, CMV, TB, Pneumocystis, Kaposis, toxoplasmosis
Disease development

- Infection to AIDS = 10 year average
- Opportunistic infections associated with T-cell destruction
- Neurotropic disease due to macrophage crossing blood brain barrier
  - Progressive mental deterioration
    - Forgetfulness, slurring of speech, dementia, fatigue, bladder/bowel incontinence
  - Neuropathy
    - Sensation of burning, numbness, pins/needles, pain in feet, legs, arms, hands
Transmission

• **Modes**
  - Transfer or introduction of infected bodily fluid, especially blood/semen

• **Routes**
  - Sexual contact, especially receptive anal sex
  - Breast milk
  - Blood-contaminated needles (tattooing)
  - Artificial insemination
  - Blood transfusion
  - Organ transplantation
History of HIV/AIDS

• Virus believed to have jumped from chimpanzees to humans some time between 1902 and 1931 in a single introduction in Leopoldville (Kinshasa) in the Democratic Republic of Congo.

• Based on genotypic analysis of two samples, one from 1959 & and other from 1960

AIDS timeline

• 1959
  - Earliest confirmed case of HIV-1 found in a blood sample of an African man who died in 1959 in Kinshasa

• 1969
  - First known case of HIV-1 in US. Teen prostitute died of Kaposi’s sarcoma and HIV

• 1981
  - Unusual immune system failure reported among gay men in US triggered by recognition of 5 men in LA with *Pneumocystis carinii* pneumonia
    - 26 gay men in NY & CA diagnosed with Kaposi’s sarcoma

• 1982
  - New disease also associated with hemophiliacs named AIDS by US health officials at CDC
    - 1300 cases of AIDS reported in US with 460 dead
AIDS timeline

• **1983**
  - At risk individuals restricted from blood donation
  - Luc Montagnier @ Pasteur Institute reports viral association with disease
  - Infection of 2 women by partners shows heterosexuals at risk
  - 4156 cases of AIDS in US, 1503 dead

• **1984**
  - Modes of transmission identified
  - HIV isolated
  - First report of infection in Thailand
  - 9,920 cases of AIDS in US, 3,498 dead

• **1985**
  - Rock Hudson discloses that he has AIDS
  - 20,470 cases of AIDS in US; 8,161 dead

• **1986**
  - 37,061 cases of AIDS in US; 16,301 dead
AIDS timeline

- **1987**
  - First Western Blot for detection is approved
  - AZT approved as first anti-AIDS drug
  - AIDS memorial quilt started in San Francisco
  - 59,572 AIDS cases in US; 27,909 dead

- **1988**
  - NIH establishes an office of AIDS research
  - 89,864 AIDS cases in US; 46,134 dead
  - 198,466 AIDS cases diagnosed in US, Death toll = 121,255

- **1995**
  - 513,486 cases in US, 43,685 deaths
  - First protease inhibitor = Saquinavir
  - First drop in deaths in US since beginning of the epidemic
AIDS timeline

• 1997
  - Worldwide reported AIDS cases from 194 countries = 1,760,000
  - Estimated HIV positive individuals worldwide= 22,000,000
  - Approximate worldwide death count=6,400,000

• 2002
  - 831,112 people in US living with HIV/AIDS
  - AIDS killed 2.4 million in sub-Saharan Africa & 3.5 million seroconverted
  - 29.4 million people in Africa are living with HIV/AIDS = 68% of those infected worldwide

• 2003
  - 40 million HIV positive individuals worldwide
  - 3.0 million deaths
AIDS timeline

• **2005**
  - 40.3 million people living with HIV worldwide
  - 4.9 million new HIV infections in 2005
  - Death due to AIDS in 2005 = 3.1 million

• **Worldwide**
  - End of 2007, 33 million people worldwide living with HIV/AIDS
  - 2.7 million new HIV infections

• **In US**
  - 2003-2007: 1,051,875 newly diagnosed cases of AIDS in US
  - More than 25 million people have died of HIV/AIDS since 1981
  - 2006: 56,300 estimated new cases of HIV

- Updated information at [http://www.globalhealthfacts.org/topic.jsp](http://www.globalhealthfacts.org/topic.jsp)
New perspectives on AIDS

• No longer considered a disease of homosexuals
  - Women increased incidence
• Extended survival in individuals as drugs and treatment improve
• Continued as major problem in Africa
  - Ratio of infected individuals male:female = 1:1
  - Widely disseminated
World AIDS Day 2004

Commemoration

Highlighting the global impact of HIV/AIDS on women and girls

Wednesday, 1 December 2004
7:30 to 9:30 p.m. [Doors open at 7:00 p.m.]

Cathedral of St. John the Divine
112th Street and Amsterdam Avenue
New York City
(1 or 2 train to 110th Street)

Co-sponsored by
Alan Guttmacher and Gloria Reshun

Keynote address by
Kofi Annan, Secretary-General of the United Nations

With musical performances by
Mara Wilson
The Louis Armstrong Quartet
and the Struthersville Choir from South Africa

Framing the personal stories of women living with
HIV/AIDS from around the world

This event is free and open to the public.

For further information, please call the
Cathedral of St. John the Divine
Box office: 212 866-2233
Images of AIDS
Antiretroviral coverage

Estimated percentage of people covered among those in need of antiretroviral treatment, situation as of November 2003.
HIV survival

• In Sub-saharan Africa
  - Average life expectancy = 47

• With ARV's
  - Average HIV positive life expectancy = 30 years from time of infection
  - Pharmaceutical companies and philanthropic organizations offering ARV's to Africa at cost
    - Some countries charge import taxes on meds, increasing the price to the patient

Source: IDSA, Oct 2007
Strategies

- **Condoms**
  - Male condom effective
  - Female condom + gel ineffective
    - Trial using antiviral gel abandoned because preliminary data showed increased transmission with gel

- **Circumcision**
  - Very promising
  - Study of discordant couples
    - Initial data suggests 100% reduction in female to male transmission if male is circumcised
      - Does not alleviate need for condoms
    - Highest density of target cells in the foreskin

- **Vaccine**
  - Most promising trial recently abandoned because control and vaccinated group had same rate of seroconversion
  - No benefit + no vaccine is completely safe

IDSA, Enders Lecture, Oct 2007
Who is HIV positive?
HSV I & II

• Enveloped dsDNA virus in Herpesviridae family
• Distinct clinical syndromes
  - Dependent on portal of entry
  - Transmission: saliva, or sexual contact
• Can become asymptomatic with continued viral shedding for years
• Clear vesicles on an erythematous base
• May have CNS manifestations
• Neonatal infections, in early childhood, also as adult
HSV and HIV Co-Infection

- Viruses work synergistically to enhance spread
  - Incr frequency of HSV in HIV infected individuals
  - Incr suscept to HIV in HSV infected
  - Incr shedding of HIV in those shedding HSV
  - Shedding of HSV and HIV decr with ganciclovir

Reported at IDSA, Oct 2007
Herpetic whitlow

- Herpes of the finger or foot
Bris

See article on bris and metzizah b’peh
Poxvirus: variola virus

• Etiological agent of smallpox

• Virus
  - Large, brick shaped
  - Complex structure

• First major infectious disease eradicated
  - >50% mortality in infected individuals
Variola virus
History of Smallpox

- 1st appeared about 2000 years ago in India
- Major epidemics (A.D.) killing $\frac{1}{4}$-$1/3$ of affected populations
  - China 49
  - Rome 165
  - Cyprus 251-66
  - Greece 312
  - Japan 552
  - Mecca 569-71
  - Arabia 683
  - Europe 700-800
smallpox
History of smallpox control

• 1709 = Jenner
• 19th century
  - Purposeful inoculation with milder form of disease
  - Mild symptoms with 1-2% fatality from inoculation
• 1949
  - Last cases of small pox in Rio Grande Valley
• 1966
  - Final WHO effort to eradicate disease
• 1979
  - World declared free of smallpox
Dr. Edward Jenner
Vaccinia vs. variola

• Smallpox vaccine was originally made from cowpox virus
• Cowpox virus is called “vaccinia virus”
• Smallpox virus is called “variola virus”
• However, at some point in 19th century, a novel orthopox virus (we still call vaccinia for historical reasons) was substituted for cowpox virus.
  - No one knows either the source or the natural reservoir
  - Some believe it exists only as a vaccine strain in the lab
  - It is still grown on excoriated cow hide.
• What is the natural reservoir for cowpox (the real vaccinia)?
  - Not cows....rats!

Orthopox viruses pathogenic to humans (2005), by Sergei Shchelkunov, et al
The vaccine and eradication efforts

• Vaccine
  - Freeze dried form requires no refrigeration
  - Inexpensive form of delivery

• What properties of virus made it possible?
  - Symptoms easily recognizable
  - No latent infection in recovering patients
  - No non-human reservoirs
  - Virus doesn’t readily mutate

• Virus kept at two reference labs
  - US, Russia
Eradication

• 1958 USSR requested international campaign to eradicate smallpox to WHO
• Eradication efforts started in 1967 led by D.A. Henderson, MD, MPH
• Involved both voluntary and forcible vaccinations
  - Had to locate foci - huge problem in 3rd world
  - House burning...reluctance to disclose
D.A. Henderson
Smallpox vaccination

- No vaccine given to the general public since 1983
- Vaccine is not without risk, though risk is relatively small
  - Contraindicated in many individuals
- Does the “fear” of a bioterrorist attack warrant vaccination of the general public?
  - What do we know?
  - Post-exposure prophylaxis (vaccine, VIG, anti-virals)
  - Engineered viruses
Small pox vaccine
Smallpox vaccination: adverse events

Eczema vaccinatum

Erythema multiforme
Smallpox vaccination: adverse events

Generalized vaccinia

Streptococcal infection
Varicella zoster virus

- Human herpesvirus 3
- Transmission
  - Droplet nuclei
- Vaccine
  - Live attenuated virus
- Treatment
  - Supportive in normal cases
  - Acyclovir in complicated cases
Shingles

Reactivation of Varicella-zoster virus
Hepatitis B Virus (HBV)

- dsDNA virus
- Transmission: bodily fluids
  - Percutaneous
  - Sexual
  - IDU
  - Blood tx

- 10% of infected children and 30-35% of infected adults are jaundiced
- Nausea, vomiting, abdominal discomfort
- Can progress to chronic (0.5% in US, > in other countries)

Remember: this is vaccine preventable!
HBV
Human Papilloma Virus

- Most common STD
  - 20 million infected in US
  - 6.2 million cases annually
- 40 serotypes
- Infects skin and mucous membranes
- Penis, vulva, vagina, cervix, anus, rectum
Consequences of HPV infection

• Not all serotypes have this effect, but some cause:
  • Genital warts
    - 1% of sexually active US adults
  • Cervical cancer
    - 2008 = 11,070 women estimated to be diagnosed
  • Other cancers
    - 3460 vulvar cancer
    - 2210 vaginal and other female genital cancers
    - 1250 penile or other male genital cancers
    - 3050 female and 2020 male anal cancers
Human Papilloma Virus

- Tree man
  - http://www.telegraph.co.uk/telegraphtv/2630624/Tree-man-before-he-underwent-treatment.html
Poliovirus

• Disease
  - Poliomyelitis

• Susceptible population
  - Small children

• Route of transmission
  - Fecal oral

• Treatment
  - Palliative

• Common vaccine
  - IPV
  - OPV
Tissue tropism of poliovirus: anterior horn of spinal cord.
Structure of Poliovirus

- Picornavirus
- Icosahedral capsid shell
Stages of infection and pathogenesis
Possible outcomes of poliovirus infection

- Asymptomatic
- Minor non-CNS illness
- Aseptic meningitis
- Paralytic
Eradication efforts

• >20 year effort
  - 1988 Global Polio Eradication Initiative comprised of WHO and partners
    • Endemic in 125 countries
    • 1000 paralytic cases in children daily
  - Currently endemic in 4 countries
    • India, Pakistan, Nigeria, Afghanistan
    • 99% reduction in cases (706 in 2010)

• Current challenges
  - Outbreaks in Angola and Tajikistan
  - Undetected asymptomatic or minimally symptomatic individuals
  - Chronic shedders of polio vaccine virus
  - Several incidents of vaccine strain reversion to neurovirulence

Clinical Infectious Disease, 12/15/10
Other viral diseases

Rubeola = measles

Koplick’s spots

Rubella = “German” measles
# Rubella vs Rubeola

<table>
<thead>
<tr>
<th></th>
<th>Rubella</th>
<th>Rubeola or morbiliivirus</th>
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<tbody>
<tr>
<td><strong>Incubation period</strong></td>
<td>2-3 weeks</td>
<td>7-18 days</td>
</tr>
<tr>
<td><strong>duration of rash</strong></td>
<td>2-3 days</td>
<td>Up to 8 days</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td>Fever 38o C or lower</td>
<td>Fever up to 40oC</td>
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<tr>
<td></td>
<td>Flu-like symptoms</td>
<td>Cough, coryza, conjunctivitis</td>
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<td>Koplick spots 3-5 days after onset (transient)</td>
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<td>Rash pink to light red, disappears after 3 days, Begins with face and spreads to trunk and limbs</td>
<td>Maculopapular, itchy, erythematous rash that stains the skin brown, Begins with face and spreads to trunk and limbs</td>
</tr>
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| **Complications**    | Congenital rubella syndrome in children of mothers exposed during first 20 weeks of pregnancy | Diarrhea, pneumonia, encephalitis, corneal ulceration, corneal scarring (most severe=subacute sclerosing panencephalitis) |
Prions

• Infectious particle of pure protein without nucleic acid implicated in several unusual neurological disease (scrapie, BSE, CJD, kuru)
  - Prion protein causes host gene to produce more copies of pathogenic protein.
  - Transmitted by injection, transplantation of contaminated tissue, electrodes, inheritable
  - New variant CJD in UK associated with BSE (1996)
Prions

- Kuru = “shivering”
  - New Guinea highlands
  - Transmission
    - Cannibalistic ceremonies of Fore Indians
    - Custom to eat bodies of deceased kinsmen
    - Increased susceptibility of women and children
  - Acquisition
    - Conjunctiva
    - Cuts in skin
    - Ingesting neural tissue
Prion diseases:
vacuolation of brain tissue
Plant viruses

- Plant viruses
  - Similar to animal viruses
  - Some capable of intracellular multiplication in insects
  - Enter through wounds in cell wall
    - Usually impermeable to viruses
    - May be assisted by nematodes, fungi, or other plant parasites
Viroids

- Short naked pieces of RNA with no protein coat
- Only found as plant pathogens, with one exception
- May be related to introns
- Some plants are deliberately inoculated with viroids as a dwarfing agent
  - Citrus viroids
Coconut cadang-cadang

- Cause of premature death in coconut palms in Philippines
- Earliest report 1914
- Killed over 12 million palms between 1926 and 1971
Hepatitis D

• Only human disease known to be caused by viroid
• Used to be called a “defective virus” = Delta agent
• Delta agent is actually a viroid enclosed in a hepatitis B capsid
• To have this disease, cells must be infected with Hepatitis B virus and hepatitis D viroid simultaneously
• Disease: causes death of liver cells
Hepatitis D (Delta) Virus

- δ antigen
- HBsAg
- RNA