Principles of clinical virology
Structure and pathogenesis

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What were the names of the Brady Bunch?
Who died of an infectious disease?
Viral Disease

- Oldest recorded disease (Rabies, polio)
- Modern epidemics/pandemics – HIV-AIDS, HCV, SARS, Avian Influenza
- Impact on - humans
  - animals
  - plants
  - evolution
1. What is a virus
   • Characteristics
   • Structure
   • Replication
   • Definitions

2. How viruses cause disease
   • Molecular principles
   • Disease pathogenesis

3. Diagnosis of viral illness
   • Principles
   • Methods

4. Clinical virology
   • principles
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A virus is a molecular genetic parasite that uses cellular systems for its own replication

Filtrable agents

Delivery system surrounding a payload
Viruses

- This intimate relationship between the virus and the cell causes several important effects:
  - Viruses are not killed by antibiotics
  - Antivirals often damage the cell
  - Viruses can persist in cells either replicating (HIV) or resting – latent (HSV)
Viruses

- Viruses are the simplest organisms, containing DNA or RNA, but not both.
- RNA viruses are more diverse and replication often error prone.
- Enveloped (environmentally unstable) and non-enveloped (environmentally stable).
Viruses have life

- Can be killed
- Can become extinct
- Undergo Darwinian selection
- Subject to evolutionary biology
But viruses

- Have no sexual exchange process
- Species is defined by its lineage
- Species is a class that occupies a replicating lineage and occupies an ecological niche
Day 1

Day 2

Day 5

Day 6

Herpes zoster
Structure

- Viruses are:
  - not cells
  - dependent upon the cell they infect. Inside cells they can replicate, outside cells they can be transmitted, but cannot replicate (grow)
  - sometimes viruses integrate their nucleic acid into the host cell genome
Virus replication
The delivery system and the payload
Virion Architecture
The delivery system

Architecture of virions regardless of host is based on two simple themes:

**Sphere** – normally in the form icosahedron (cubical)
- Best way of producing a shell of equivalently bonded identical structures
- Minimum free energy state
- Strong structure that can enclose a maximal volume

**Helix** – cylindrical shape (spiral staircase)
Virion Architecture – icosahedron

An ICOSAHEDRON is composed of 20 facets, each an equilateral triangle, and 12 vertices (corners)
Helical viral structure

Several RNA viruses undergo self assembly as a cylindrical nucleocapsid. (*hollow tube*)

The viral RNA forms a spiral within the capsid structure

Each capsomer consists of a single protein
Examples of virus structure
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Viruses as a molecule
The payload

- Most viruses
  - 10 – 20 genes
  - Genomes 5,000 – 25,000 bp

- ICTVdb
  - 3,600 species
  - 30,000 strains + subtypes
Viruses as a molecule

- ssRNA – most diverse Noro, HCV, HIV
- dsDNA – Adeno, CMV, HSV, Variola
- dsRNA – Rota
- ssDNA – least diverse PVB19
Acute and persistent virus life strategies

- No persistence in individual host
- Often disease associated
- High mutation rates (RNA viruses)
- Virus replicates in more than one species
- Little coevolution with host
- Horizontal transmission
- Highly dependent on host population structure
- Seldom evolves to persistence
Acute and **persistent** virus life strategies

- Persistent in individual host
- Acute disease often inapparent
- Genetically stable
- Highly species specific
- Coevolution with host
- Transmission is often from parent to offspring (vertical) or through sexual contact
- Less dependent on host population structure
- Often the source of emerging acute disease in new host species
Classification

- Viral nucleic acid + virus capsid + envelope
- Other characteristics:
  - Genomic makeup e.g.: Caliciviruses
  - Virion structure – EM appearance e.g.: herpes
  - Replication strategy
  - Virion antigenicity e.g.: adenoviruses, serological distinction MVE / JE / WNV
  - Virion chemical characteristics, stability
  - Diseases caused in the host e.g.: hepatitis
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Diagnostic Methods

- Serology - retrospective
- Ag
  - Protein based
  - IFA (Respiratory)
  - WB (HIV)
  - Protein function (HIV-RT)
- Culture – some viruses non-cultivable
- Molecular
  - Virion nucleic acid
  - HIV RNA, HCV RNA
  - CMV DNA
- Emerging Microarray different formats, HPLC, Protein amplification, MALDI-TOF
Serology

- ELISA, IFA, CFT
- Total (EV, HAV, HCV, HW, Influenza)
- IgM (Adeno, HSV)
- IgG (CMV, Mumps, Measles, PVB19 Rubella)
- Complex (HBV, EBV)
- No use (Rota, Noro, Variola)
Molecular testing

- Rapid, sensitive, costly (initially)
- Amplified
  - Target (PCR, LCR, NASBA, TMA)
  - Signal (bDNA)
- Non amplified
  - Probe based (ISH, Hybrid Capture)
Multiplex PCR Configurations

- Organ system specific
- Liver
- GIT
- Symptom specific
- Age of patient
- Epidemiological characteristics of pathogens
- Syndrome specific
- Screening
<table>
<thead>
<tr>
<th>VDL01 DNA agents</th>
<th>VDL05 Common pathogens</th>
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<tbody>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>HSV-1 and -2</td>
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<tr>
<td>HSV-1 and -2</td>
<td>CMV</td>
</tr>
<tr>
<td>CMV</td>
<td>Varicella-zoster virus</td>
</tr>
<tr>
<td>Parvovirus</td>
<td>Epstein-Barr virus</td>
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<tr>
<td>Varicella-zoster virus</td>
<td>Enterovirus</td>
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<thead>
<tr>
<th>VDL STI</th>
<th>VDL STI</th>
<th>VDL STI</th>
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<tbody>
<tr>
<td>U. parvum</td>
<td>C. trachomatis</td>
<td>HSV-1</td>
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<tr>
<td>U. urealyticum</td>
<td>T. vaginalis</td>
<td>HSV-2</td>
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<tr>
<td>M. hominis</td>
<td>T. pallidum</td>
<td>VZV</td>
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<tr>
<td>M. Genitalum</td>
<td>GBS</td>
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</tbody>
</table>
Ag testing

- Existing technique (Respiratory)
- Initial testing (p24)
- Only available (Prion)
- Dependent upon
  - many host cells in specimen
  - operator
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Viral Developments

- New pathogens identified
  - Coronaviruses SARS, NL63, HKU1
  - Parvoviruses bocavirus, PARV4
  - Retroviruses XRMV in CFS
  - Arenavirus in transplantation
  - Mimivirus *Acanthamoeba polyphaga mimivirus* (APMV)
  - Adenovirus 14 in pneumonia
Viral Developments

- New intraspecies transmission
  - H5N1
  - Sin Nombre/hantaviruses
  - Nipah
  - Ebola
  - Arenavirus
  - nvBSE
  - HIV
  - PERV

- Transmission without clear diseases
  - Spumaretorviruses
  - Reovriuses
  - Adeno associated parvoviruses
  - TT virus
Viral Developments

- New diseases of old pathogens
  - hMPV
  - HRV
- Extended spread of known diseases
  - Chikungunya
  - Dengue
- Documentation of chronic diseases
  - HIV
  - HCV occult
  - HBV occult
Viral Syndromes

- Adenopathy and glandular fever
- Arthritis
- Carditis
- Chronic Fatigue Syndrome
- Congenital and perinatal disease
- Exanthemata and skin disease
- Eye disease
- Gastroenteritis
Viral Syndromes

- Haemorrhagic fevers
- Hepatitis
- Immunocompromised infections
- Neurological disease
  - encephalitis and meningitis
- Pancreatitis and diabetes
- Respiratory disease
- Sexually Transmitted Infections (STD, STI)
Agents of risk
Known / tested

- HBsAg  - Sept 1970
  - Abbott PRISM
- HCVAb  - Feb 1990
  - Abbott PRISM
- HIV 1/2 Ab - April 1985
  - Abbott PRISM
- HTLV I/II Ab - Jan 1993
  - Abbott PRISM
- HCV RNA - June 2000
  - Pooled 24, TMA Chiron
  - Pooled 16, April 2005
- HIV 1 RNA - June 2000
  - Pooled 24, TMA Chiron
  - Pooled 16, April 2005
- CMV - selected
Agents of risk
Known / not tested

- CMV
- GB-C virus - unknown
- HHV8
  - few donors
  - theoretical KS
- Prions vCJD
  - few donors
  - transmissible encephalopathy
- SARS coronavirus - few donors
- TT virus – 80-95% of donors
- SEN-V
  - 2% of donors
  - types A-H
  - hepatitis
- WNV
  - few donors
  - 3 week deferral process
  - encephalitis
Agents of risk
Known / emerging risk

- Dengue - deferral procedures for at-risk
- Prions - vCJD [Llewellyn, 2004]
  - exclusion
- SARS coronavirus
- WNV
- Unknown
  [ Chamberland, 2001]
Carol Brady
Mike Brady
Greg Brady
Marcia Brady-Logan
Peter Brady
Jan Brady
Cindy Brady
Alice Nelson

Tiger the dog
Fluffy the cat

and Mike Brady (Robert Reed) died of AIDS in 1992
and Marcia Brady (Maureen McCormick) grandmother died of syphilis
- Poxviruses
  - Variola (smallpox)
  - Myxoma
  - Ectromelia (mousepox)
- Infectious diseases
  - Malaria
  - TB
- Viral taxonomy
- Microbiology history
- Prime Minister’s Prize (2002)
- Royal Society Copley medal (1996)
- Japan Prize for Preventive Medicine (1988)
Epidemics

- Measles
  - Plague of Athens (436 B.C.) described a distemper-like epidemic with high mortality
  - Epidemics - Rome, China AD165, AD251
  - Considered a normal process of development
  - All adults survivors of childhood infection in Europe
Epidemics

- **Smallpox**
  - Earliest accounts from India, in Sanskrit medical text, China, 1122 B.C.
  - Entered Europe via Islamic North African expansion to Spain; epidemics in Syria (A.D. 302) and Mecca (A.D. 569)
  - Reintroduced to Europe via crusaders
  - Disease milder limited to children (Spain in the 1400s)
VIRAL INFECTIONS CHILDREN

Total Specimens

Month

- Negative
- FluA
- hRSV
- PIV3
- hMPV

Virology Division
Rotavirus 2003 (n = 1701)
Rotavirus 2004 (n = 1514)
Rotavirus 2005 (n = 1346)
Norovirus 2004 (n = 1130)
Norovirus 2005 (n = 298)
Congenital CMV Infection

- Hepatosplenomegaly
- Jaundice
- Microcephaly
- Prematurity
- Chorioretinitis
- Petechiae
- Mental retardation
- Hearing loss
Normal (uninfected) placental tissue
Blood Supply testing

- Mainly viral
- Ignores persistent non-threatening viruses
- Constantly changing
- Role of emerging viruses
<table>
<thead>
<tr>
<th>HEPATITIS VIRUS</th>
<th>ACUTE HEPATITIS</th>
<th>CHRONIC HEPATITIS</th>
<th>FULMINANT HEPATITIS</th>
<th>CIRRHOSIS</th>
<th>HCC</th>
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<tbody>
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<td>+</td>
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Some Emerging Issues

- New respiratory virus – SARS
  - hMPV
  - Associations with chronic conditions
- Transfusion – HCV
  - Non A non B non C
  - New viruses (GBV, TTV, Sen V)
- Old viruses re-emerging
  - Smallpox
- Zoonoses
  - SARS
  - Rabies
  - Arenaviruses
  - Hantaviruses
What are the names of the seven dwarves, and what was the woman.
What are the names of the seven dwarves, and what was the woman.

Dopey, Grumpy, Doc, Happy, Bashful, Sneezy, Sleepy

An only child, maternal death, somnolence, hallucinations
Queen you are full fair, ‘tis true, but Snow White is fairer than you
The fairest of them all