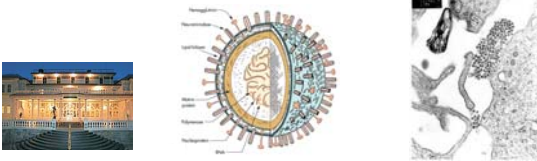


Principles of clinical virology

Structure and pathogenesis



Bill Rawlinson

Virology Division, SEALS Microbiology

August 2012



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1. What is a George Bush virus?

OUTLINE



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1. What is a George Bush virus?

OUTLINE

One that keeps looking for viruses of mass destruction



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Viral Disease

- Oldest recorded disease (Rabies, polio)
- Modern epidemics/pandemics – HIV-AIDS, HCV, SARS, Avian Influenza
- Impact on
 - humans
 - animals
 - plants
 - evolution



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1. What is a virus

OUTLINE

- Characteristics
- Structure
- Replication
- Definitions
- 2. How viruses cause disease
 - Molecular principles
 - Disease pathogenesis
 - Koch's postulates and viral pathogenesis
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 - Principles
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 - 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



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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)



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Viruses have life

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



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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



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Day 1



Day 2



Day 5



Day 6



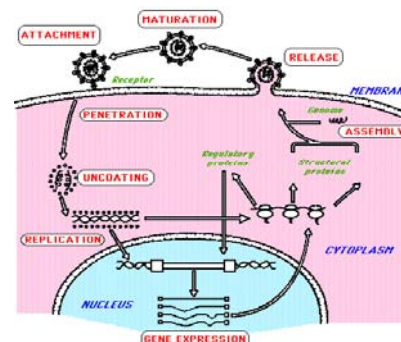


Structure

- 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

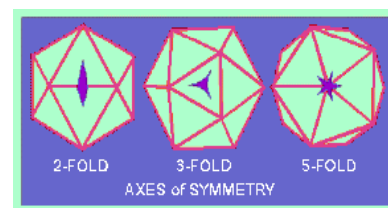
Architecture of virions regardless of host is based on two simple themes:

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 **ICOSAHDREDON** is composed of 20 facets, each an equilateral triangle, and 12 vertices (corners)

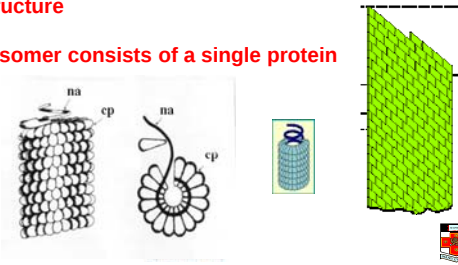


Helical viral structure

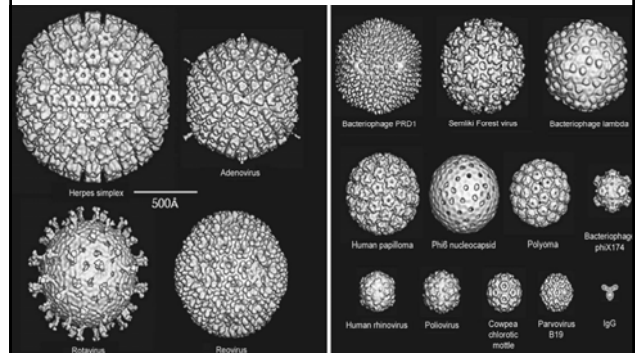
Several RNA viruses undergo self assembly as a cylindrical nucleocapsid. (*hollow tube*)

The viral RNA forms a spiral within the capsid structure

Each capsomer consists of a single protein



Examples of virus structure



Viruses as a molecule - A payload

- Most viruses
 - 10 – 20 genes
 - Genomes 5,000 – 25,000 bp
- ICTVdb
 - 6 orders
 - 87 families, 19 subfamilies
 - 349 genera
 - 2284 virus and viroid species
 - 30,000 strains + subtypes



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Viruses as a molecule

- ssRNA – most diverse Noro, HCV, HIV
- dsDNA – Adeno, CMV, HSV, Variola
- dsRNA – Rota
- ssDNA – least diverse PVB19



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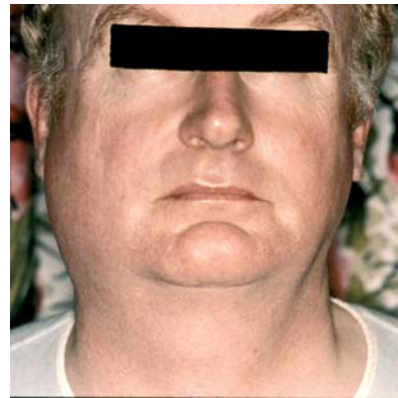


Acute and persistent virus life strategies

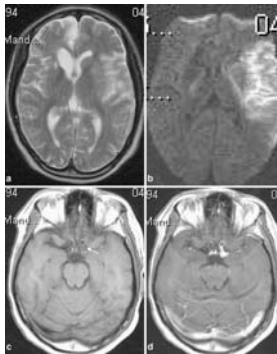
- No persistence in individual host
- Often disease associated
- High mutation rates (RNA viruses)
- Virus replicates in more than one species
- Little coevolution with host
- Horizontal transmission
- Highly dependent on host population structure
- Seldom evolves to persistence



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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



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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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Koch's stamp



Koch's statue



Koch's house




Robert Koch




Koch's postulates



- To establish that an organism is the cause of a disease, it must be:
 - found in all cases of the disease examined, while absent in healthy organisms
 - prepared and maintained in a pure culture
 - capable of producing the original infection, even after several generations in culture
 - retrievable from an inoculated animal and cultured again





VIRUSES AND KOCH'S POSTULATES'

THOMAS M. RIVERS

From The Rockefeller Institute for Medical Research, New York

Diseases at one time were thought to be caused by wrath of the gods, configuration of stars or miasmas. After a real struggle that occurred not so many years ago, certain maladies were shown to be induced either by small animals or minute plants, e.g., protozoa, fungi, bacteria and spirochetes. Indeed, the victory was so great that most workers in time began to consider that all infectious diseases, including those whose incitants had not been discovered, must be caused by agents similar to those already recognized. According to them, there could be no infections that were not caused by protozoa, fungi, bacteria or spirochetes, and to intimate that some infectious agents might be inanimate constituted heresy of the first order.

¹ Presidential address delivered before the Society of American Bacteriologists at its Thirty-eighth Annual Meeting, Indianapolis, Indiana,

December 29, 1936

JOURNAL OF BACTERIOLOGY, VOL. 33, NO. 1




Koch's organisms

- Pupils found the organisms responsible for
 - [diphtheria](#)
 - [typhoid](#)
 - [pneumonia](#)
 - [gonorrhoea](#)
 - [leprosy](#)
 - [plague](#)
 - [tetanus](#)
 - [Syphilis](#)
 - etc





Koch's competitors



- Founders of modern microbiology
 - Ferdinand Cohn
 - Louis Pasteur
 - Robert Koch
- Pasteur's work
 - Vaccines anthrax, rabies
 - Puerperal fever
 - Pasteurization of milk






Koch's postulates 2012



- Clearly different postulates in viruses
 - **Difficulties with disease associations**
 - TTvirus hepatitis
 - Human Bocavirus respiratory
 - SARS coronavirus SAR syndrome
 - **Problems**
 - Convenience samples
 - Bias case ascertainment, illness severity
 - Inadequate controls
 - Syndromic illnesses
 - NAT sensitive, coinfection, viral load, prolong shedding





Revised postulates - Huebner



- Guarantee against imputation by association
 - Isolation in culture
 - Repeated recovery from humans
 - Specific Ab response
 - Comparison with known pathogenic viruses
 - Constant association with illness
 - Reproduce illness in volunteer studies
 - Epidemiologic/longitudinal studies
 - Prevention of disease by vaccination



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[Huebner 1957]



Revised postulates – Fredericks and Relman



- Need for revision of postulates based on sequence
 - Sequence should be present in most cases, preferentially in tissues known to be diseased
 - Fewer/no copies in non-diseased hosts and tissues
 - Disease resolution results in decreased/no sequences
 - Sequence detection predates disease, viral load copy number correlates with disease/pathology severity
 - Nature of organism inferred from sequence consistent with biology of the genus, and clinical phenotype
 - Tissue-sequence correlates sought
 - Above all reproducible



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[Fredericks 1996]



Koch's roosters



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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



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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)



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Molecular testing

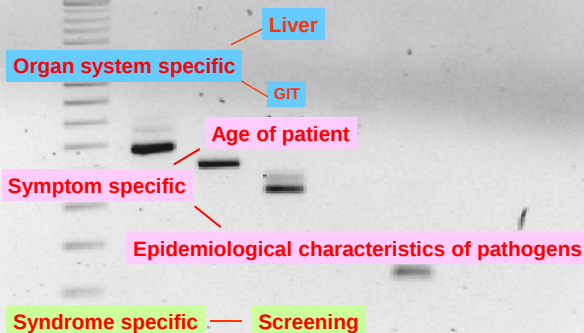
- Rapid, sensitive, costly (initially)
- Amplified
 - Target (PCR, LCR, NASBA, TMA)
 - Signal (bDNA)
- Non amplified
 - Probe based (ISH, Hybrid Capture)



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Multiplex PCR Configurations



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



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Viral Developments

- New intraspecies transmission
 - H5N1
 - Sin Nombre/hantaviruses
 - Nipah
 - Ebola
 - Arenavirus
 - nvBSE
 - HIV
 - PERV
- Transmission without clear diseases
 - Spumaretoviruses
 - Reoviruses
 - Adeno associated parvoviruses
 - TT virus



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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



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Viral Syndromes

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



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Viral Syndromes

- Haemorrhagic fevers
- Hepatitis
- Immunocompromised infections
- Neurological disease
 - encephalitis and meningitis
- Pancreatitis and diabetes
- Respiratory disease
- Sexually Transmitted Infections (STD, STI)



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d'Affinois



d'Affinois

Cantal vieux



d'Affinois

Cantal vieux

Selles sur Cher



d'Affinois

Cantal vieux

Selles sur Cher

Tomme de Savoie



d'Affinois

Cantal vieux

Tomme de Savoie

Saint Andre





- OUTLINE**
1. What is a George Bush virus?
 - Causes your computer to keep looking for viruses of mass destruction
 2. The Ronald Reagan virus?



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- OUTLINE**
1. What is a George Bush virus?
 - Causes your computer to keep looking for viruses of mass destruction
 2. The Ronald Reagan virus?
 - Saves your data, but forgets where it is stored



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- OUTLINE**
1. What is a George Bush virus?
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 3. The Mike Tyson Virus?



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- OUTLINE**
1. What is a George Bush virus?
 - Causes your computer to keep looking for viruses of mass destruction
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 - Saves your data, but forgets where it is stored
 3. The Mike Tyson Virus?
 - Quits after two bytes



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- OUTLINE**
1. What is a George Bush virus?
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 - Saves your data, but forgets where it is stored
 3. The Mike Tyson Virus?
 - Quits after two bytes
 4. Jack Kevorkian Virus?
 - Deletes all old files



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Agents of risk Known / tested

- HB_eAg - Sept 1970 - Abbott PRISM
- HCV RNA - June 2000 - Pooled 24, TMA Chiron - Pooled 16, April 2005
- HCVAb - Feb 1990 - Abbott PRISM
- HIV 1 RNA - June 2000 - Pooled 24, TMA Chiron - Pooled 16, April 2005
- HIV 1/2 Ab - April 1985 - Abbott PRISM
- HTLV I/II Ab - Jan 1993 - Abbott PRISM
- CMV - selected



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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



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Agents of risk Known / emerging risk

- Dengue - deferral procedures for at-risk
- Prions - vCJD [Llewellyn, 2004]
 - exclusion
- SARS coronavirus
- WNV
- Unknown

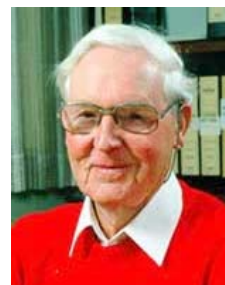
[Chamberland, 2001]



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- 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)



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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



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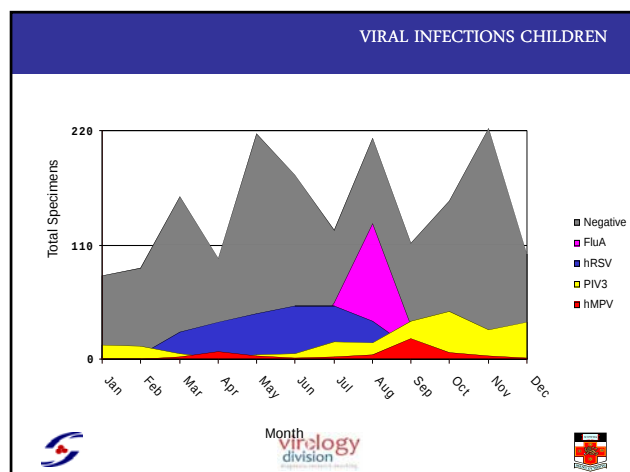
Epidemics

• 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)



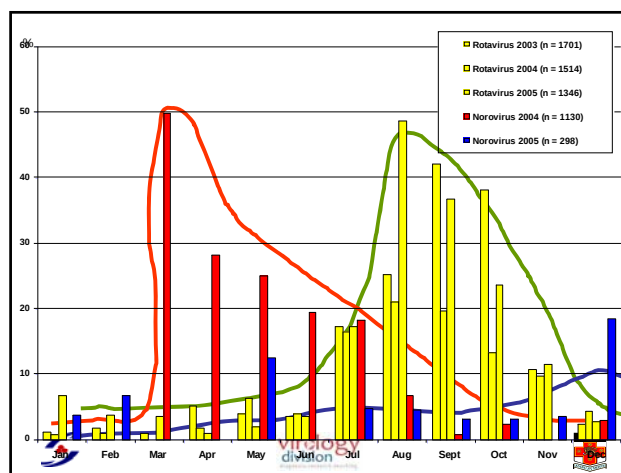
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Congenital CMV Infection

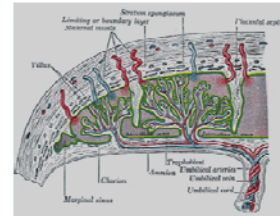
- Hepatosplenomegaly
- Jaundice
- Microcephaly
- Prematurity
- Chorioretinitis
- Petechiae
- Mental retardation
- Hearing loss



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Normal (uninfected) placental tissue



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Blood Supply testing

- Mainly viral
- Ignores persistent non-threatening viruses
- Constantly changing
- Role of emerging viruses



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HEPATITIS VIRUS	ACUTE HEPATITIS	CHRONIC HEPATITIS	FULMINANT HEPATITIS	CIRRHOSIS	HCC
A	+	0	+	-	-
B	+	5-10%	+	+	+
C	+	75%	+	+	+
D	+	<5% 50%	+	+	+
E	+	0	+	-	-
G	+	20%	-	-	-
TTV	+	60%	-	-	-



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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

