Introduction to Virology

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Landmarks in Virology

• Introduction of concept of ‘filterable agents’ for plant pathogens (Mayer, Ivanofsky, Beijerinck in late 1880’s)
• First filterable agent from animals described – foot and mouth disease virus (Loeffler and Frosch in 1898)
• First human filterable agent described - yellow fever virus (Reed in 1901)
• Linkage of viruses with cancer (Ellerman, Bang 1908; Rous 1911)
Landmarks in Virology

• Description of bacteriophages (Twort and D'Herelle in 1915)
• Visualization of viruses by EM and x-ray crystallography (1939, 1941)
• Development of tissue culture systems (Sanford, Enders, Gay, Eagle 1948-1955); growth of poliovirus in culture
• Discovery of many agents; explosion in molecular biology (past 50+ years)

‘Virus’

Latin for ‘slimy liquid’ or ‘poison’
Definitions

• Virus particle or virion
  - Infectious agent composed of nucleic acid (RNA or DNA), a protein shell (capsid) and, in some cases, a lipid envelope

• Capsid
  - Protein coat that surrounds the viral nucleic acid
  - Composed of repeating subunits called capsomeres
  - Have either icosahedral or helical symmetry

• Nucleocapsid
  - Complete protein-nucleic acid complex

Definitions

• Satellite or defective viruses
  - Viruses which require a second (helper) virus for replication
    » Example: hepatitis delta virus requires hepatitis B

• Viroids
  - Small, autonomously replicating molecules
  - Single stranded circular RNA, 240-375 residues in length
  - Plant pathogens

• Prions
  - Not viruses
  - Infectious protein molecules responsible for transmissible and familial spongiform encephalopathies
    » e.g., Creutzfeldt-Jakob disease, bovine spongiform encephalopathy (vCJD in humans)
  - Pathogenic prion protein PrP\textsuperscript{Sc} formed from normal human protein, PrP\textsuperscript{C}, through post-translational processing
Virus Classification

• Older based on
  - Host, target organ or vector
• Modern based on
  - Type of viral nucleic acid
    » RNA or DNA
    » Single stranded (SS) or double stranded (DS)
    » Replication strategy
  - Capsid symmetry
    » Icosahedral or helical
  - Presence or absence of lipid envelope
• Governed by International Committee on Taxonomy of Viruses

Capsid Symmetry

Icosahedral   Helical
Virion Morphology

![Virion Morphology Diagram]

From Principles and Practice of Infectious Diseases

Virus Classification

<table>
<thead>
<tr>
<th>Family</th>
<th>Example</th>
<th>Type of Nucleic Acid</th>
<th>Genome Size (Ribbons or Kilobase Pairs)</th>
<th>Envelope</th>
<th>Capsid</th>
<th>Sensitivity</th>
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<tbody>
<tr>
<td>RNA-containing Viruses</td>
<td>Poliovirus</td>
<td>SS + RNA</td>
<td>7.5-8.4</td>
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<td>Rabies virus</td>
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<tr>
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<td>Hepatitis A virus</td>
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<td>7-11</td>
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<td>Circular ds DNA with SS proteins</td>
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<td>Circular ds DNA with SS proteins</td>
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</table>

1: ss = single stranded, ds = double stranded, circular = circular, ss = single stranded, *= 1 segment, #= 12 segments, **= 7 segments, ***= 11 segments, ****= 13 segments, *****= 15 segments.

From Principles and Practice of Infectious Diseases
Viruses: Basic Concepts

- Viruses are obligate intracellular parasites
- Viruses carry their genome (RNA or DNA) and sometimes functional proteins required for early steps in replication cycle
- Viruses depend on host cell machinery to complete replication cycle and must commandeer that machinery to successfully replicate

Viral Replication: Basic Concepts

- Replication cycle produces
  - Functional RNA's and proteins
  - Genomic RNA or DNA and structural proteins
- 100’s-1,000’s new particles produced by each cycle
  - Referred to as burst size
  - Many are defective
  - End of 'eclipse' phase
- Replication may be cytolytic or non-cytolytic
Coronavirus

Family: Coronaviridae
(+) SS RNA, enveloped, helical

SARS

- Etiology:
  - Newly described coronavirus
    - Fully sequenced by two groups within a few weeks after isolation

- Origin
  - Perhaps cross-species infection and viral recombination

- Power of information and laboratory technologies highlighted by this outbreak

- Globalization of infectious disease outbreaks and economic impact also highlighted
Coronavirus

- Member of the Coronaviridae family
- Pleomorphic 100-150 nm particle with characteristic surface projections
  - Single stranded, (+) sense RNA genome (27-32 kb)
  - Cytoplasmic replication
  - Viral assembly in Golgi apparatus and endoplasmic reticulum
- Infects multiple species
  - Chickens, turkeys, mice, rats, cats, dogs, rabbits, cattle, pigs and humans
- In humans
  - Before SARS – clinical expression was mild respiratory disease in healthy persons
  - Gastrointestinal disease?
- Respiratory illness has been seasonal
  - Peaks in winter and spring
- In volunteer studies
  - Virus shed for 48 h after inoculation and continues for approx. 5 d

Spread from Hotel M
Reported as of March 28, 2003
SARS - 2003

- Human cases date back to November 2002 in China
- Local chains of transmission reported in mainland China, Hong Kong, Taiwan, Hanoi, Singapore, Toronto, UK and US
- 8,096 cases in 29 countries
- 774 deaths
  - Case fatality rate 9.6%

SARS: ?Origin

Guangzhou Food Market  Civet
SARS: Clinical Description

- Incubation period 2 – 7 days
  - Maybe as long as 10 days
- Illness begins with prodrome of fever
  - Chills, headache, malaise, myalgia, diarrhea may also be present
- Next phase: dry cough and/or shortness of breath
- In 10-20% disease may be rapidly progressive and require mechanical ventilation
- Chest films: normal → focal interstitial infiltrates → more generalized infiltrates → consolidation and ARDS
- Lymphopenia, thrombocytopenia, elevated CPK and hepatic enzymes may be seen
- Treatment is supportive
- Full spectrum of disease unknown
SARS: Diagnosis

- **Clinical suspicion**
  - Particularly in a traveler from an endemic region or someone exposed to a possible/probable case

- **Laboratory**
  - Still investigational
  - Sputum, blood and body fluids for viral cultures and PCR
  - Antibody
    - May not be positive for up to 28 days

SARS: Radiographic Characteristics

NEJM: 2003
SARS Coronavirus Excretion

Figure 4: Sequential quantitative RT-PCR for SARS-associated coronavirus in nasopharyngeal aspirates of 14 SARS patients

Paramyxovirus

Family: Paramyxoviridae
(-) SS RNA, enveloped, helical
Measles

- Measles virus is a member of the Paramyxoviridae family, genus Morbillivirus
  - Primates are the only natural hosts
- Classically a childhood illness, spread by the respiratory route
  - Primary and secondary viremia
- Incubation period is 10-14 days, followed by 2-3 day prodrome of fever, cough, coryza and conjunctivitis
  - Koplik spots in pharynx may appear
- Maculopapular rash follows
  - Temporally associated with beginning of viral clearance
  - Starts on face and behind ears; moves centrifugally
  - Typically, clinical improvement as rash resolves

Measles

- Complications
  - Pneumonia (giant cell)
  - Encephalitis
  - Subacute sclerosing panencephalitis (SSPE)
    - Rare in vaccine era, but seen years after measles acquired at an early age (≤2)
      - High titers of anti-measles Ab
  - Ocular
  - Atypical measles
    - Seen in persons exposed to natural measles virus following vaccination with killed vaccine years earlier
- Mortality can be high in malnourished and immuno-compromised populations
- Despite presence of an effective vaccine, 30 million cases reported worldwide in 2003 with 530,000 deaths
  - >95% in countries with per capita income <$1000/yr
  - Seen in US by importation or in unvaccinated persons
- Vaccine preventable
  - Live attenuated vaccine
Measles

Rash

Koplik spots

Giant cell pneumonia

Influenza Virus

Family: Orthomyxoviridae

(-) SS RNA segmented, enveloped, helical
Ebola Virus

- Family: Filoviridae
- (-) SS RNA, enveloped, helical

Rotavirus

- Family: Reoviridae
- DS RNA segmented, nonenveloped, icosahedral
Retroviruses

Family: Retroviridae
2 identical (+) RNA strands, enveloped, icosahedral capsid, helical nucleoprotein

Hepatitis B Virus

Family: Hepadnaviridae
Circular DS DNA with SS portions, enveloped, icosahedral
Parvovirus

Family: Paroviridae
SS DNA, nonenveloped, icosahedral

B19 Parvovirus: Erythema Infectiosum

From Clinical Virology
Papillomavirus

Family: Papovaviridae
Circular DS DNA, nonenveloped, icosahedral

Cutaneous Wart

From Clinical Virology
Adenovirus

Family: Adenoviridae
Linear DS DNA, nonenveloped, icosahedral
Adenovirus Conjunctivitis

From Clinical Virology

Adenovirus Tonsillitis

From Clinical Virology
Herpesvirus

Family: Herpesviridae
Linear DS DNA, enveloped, icosahedral

Herpes Simplex Virus Keratitis

From Clinical Virology
Cytomegalovirus Retinitis

From Clinical Virology

Poxvirus

Family: Poxviridae
Linear DS DNA, enveloped, complex
Smallpox

Viral Pathogenesis:
Elements of Virus-Host Interaction

- Viral strain
- Inoculum size
- Route of exposure
- Susceptibility of host
  - Is there pre-existent immunity from past exposure or vaccination?
  - Host genetic factors
- Immune status and age of host
Viral Pathogenesis:
Net Result of Virus-Host Interaction

• No infection
• Abortive infection with limited viral replication
• Asymptomatic infection
• Symptomatic infection
• Persistent, latent or self-limited infection
  - Depending upon the agent and immune competence of host
• Influenced by availability of effective prophylaxis or therapy

Pathogenetic Steps in Human Viral Infection

• Virus may enter through skin, mucous membranes, respiratory tract, GI tract, via transfusion, needle-stick, or maternal-fetal transmission
• Local replication at site of inoculation
  - Certain agents may cause pathology here
• Neurotropic agents may travel along nerve routes or reach CNS by viremic spread
Pathogenetic Steps in Human Viral Infection

- For many agents, there is replication in regional lymph nodes with subsequent viremia and spread to target organs
  - Some travel free in plasma (e.g., picornaviruses); some are cell associated (e.g., cytomegalovirus)
- Replication in target organs may lead to local damage and further viremia
- Non-specific and virus-specific host immune responses come into play to downregulate viral replication

Immune Response to Viral Infections

- Innate (non-specific) immunity
  - Phagocytic cells (neutrophils and monocyte-macrophages)
  - Cytokines (e.g., interferons) and chemokines
  - Natural killer cells
  - Other 'antiviral' factors
- Adaptive (specific) immunity
  - Antigen specific B and T cell responses
    » Antibodies
    » Cytotoxic T cells
    » Antibody dependent cellular cytotoxicity
- Immunopathologic injury
Viral Persistence

- Viruses may cause chronic, persistent infection in the face of an immune response
  - HIV, hepatitis B, hepatitis C

- Immune compromise may result in persistent infection where latency or elimination may have otherwise occurred
  - Herpesviruses, papillomaviruses, rubella virus

Viral Persistence

- Some viruses cause latent infection
- Latency is characterized by a quiescent or minimally transcriptionally active viral genome with potential periods of reactivation
  - Herpesviruses
  - Human retroviruses
  - Human papillomaviruses
- Viruses which exhibit latency may also exhibit chronic, persistent infection in the setting of immune compromise
Viral Persistence

• Mechanisms
  - Persistent/chronic infection
    » Antigenic variation to escape antibody or CTL responses
    » Downregulation of class I major histocompatibility antigens
    » Modulation of apoptosis
    » Privileged sites
  - Latency
    » Decreased viral antigen expression and presentation to the immune system

Viral Persistence

• Sites
  - Nervous system
    » Herpes simplex virus, varicella-zoster virus
    » JC virus
    » Measles virus
  - Liver
    » Hepatitis B virus, hepatitis C virus, hepatitis D virus
  - Leukocytes
    » HIV, cytomegalovirus, Epstein-Barr virus
  - Epithelial tissue
    » Papillomaviruses
Oncogenesis: Associations

- Epstein-Barr virus with lymphoma, nasopharyngeal carcinoma and leiomyosarcoma
- Herpesvirus 8 with Kaposi’s sarcoma and body cavity B-cell lymphoma
- Hepatitis B and C viruses with hepatocellular carcinoma
- Human papillomavirus with cervical cancer and anogenital carcinoma
- HIV with Kaposi’s sarcoma and lymphoma via immunosuppression

Diagnosis of Viral Infections

- Clinical suspicion
  - Is syndrome diagnostic of a specific entity?
  - Is viral disease in the differential diagnosis of a presenting syndrome?
- Knowledge of appropriate specimen(s) to send
  - Blood
  - Body fluids
  - Lesion scraping
  - Tissue
  - Proper transport is essential
Diagnosis of Viral Infections

- Isolation of virus in tissue culture, animals, embryonated eggs
- Antigen detection in body fluids, blood, lesion scrapings, or tissue
- Nucleic acid detection in body fluids, blood or tissues
- Antibody detection
  - Presence of IgM or 4-fold rise in IgG titer
- Tissue biopsy for light microscopy supplemented by antigen and/or nucleic acid detection
- Electron microscopy of body fluids or tissues

Viral Infections: Prevention and Therapy

- Vaccines
  - One of the most significant advances in human health
    » Eradication of smallpox is prime example
  - Effective vaccines exist for polio, mumps, measles, rubella, influenza, hepatitis A, hepatitis B, varicella-zoster, rabies, adenovirus, Japanese B encephalitis, yellow fever, smallpox, human papillomavirus
- Immune globulin for prevention or amelioration of clinical disease
  - Varicella-zoster immune globulin, rabies immune globulin, cytomegalovirus immune globulin, respiratory syncytial virus immune globulin and palivizumab, immune serum globulin for hepatitis A
Viral Infections: Prevention and Therapy

- **Blood screening**
  - HIV-1, HIV-2, HBV, HCV, HTLV-1, HTLV-2
  - In certain settings
    » West Nile Virus
    » CMV

- **Safe sexual practices**
  - HIV, hepatitis B, HSV, and human papillomavirus infections

- **Specific antiviral therapy**
  - Herpes simplex virus, varicella-zoster virus, cytomegalovirus, HIV, influenza virus, respiratory syncytial virus, hepatitis B and hepatitis C