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

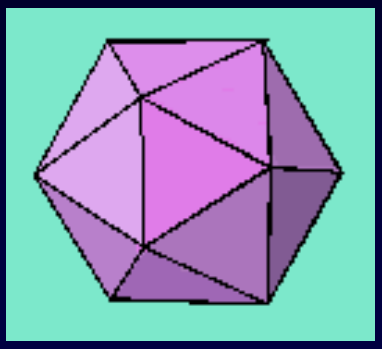
Definitions

- 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^{Sc} formed from normal human protein, PrP^{C}, through post-translational processing

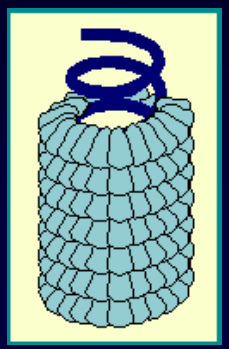
Virus Classification

- 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 symmety
    - » Icosahedral or helical
  - Presence or absence of lipid envelope
- Governed by International Committee on Taxonomy of Viruses

Capsid Symmetry

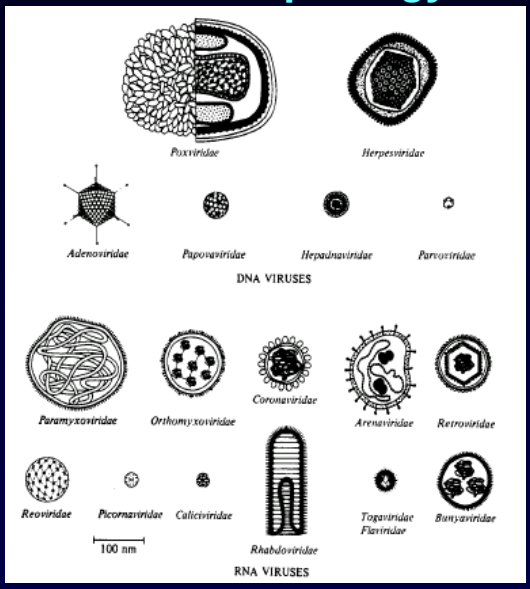


Icosahedral



Helical

Virion Morphology



From ***Principles and Practice of Infectious Diseases***

Virus Classification

TABLE 119–1 Classification of Viruses

Family	Example	Type of Nucleic Acid	Genome Size (Kilobases or Kilobase Pairs)	Envelope	Capsid Symmetry
RNA-containing viruses					
Picornaviridae	Poliovirus	SS (+)RNA	7.2–8.4	No	I
Caliciviridae	Norwalk virus	SS (+)RNA	7.4–7.7	No	I
Astroviridae	Astrovirus	SS (+)RNA	7.2–7.9	No	I
Togaviridae	Rubella virus	SS (+)RNA	10–12	Yes	I
Flaviviridae	Yellow fever virus	SS (+)RNA	9.5–13	Yes	Unk
Coronaviridae	Coronavirus	SS (+)RNA	20–30	Yes	H
Rhabdoviridae	Rabies virus	SS (−)RNA	13–16	Yes	H
Filoviridae	Ebola virus	SS (−)RNA	19	Yes	H
Paramyxoviridae	Measles virus	SS (−)RNA	16–20	Yes	H
Orthomyxoviridae	Influenza virus	8 SS (−)RNA segments*	10–14	Yes	H
Bunyaviridae	California encephalitis virus	3 circular SS (ambisense) RNA segments	11–21	Yes	H
Arenaviridae	Lymphocytic choriomeningitis virus	2 circular SS (ambisense) RNA segments	10–14	Yes	H
Reoviridae	Rotavirus	10–12 DS RNA segments†	16–27	No	I
Retroviridae	Human immunodeficiency virus type 1	2 identical SS (+)RNA segments	7–11	Yes	I-capsid H-nucleocapsid
DNA-containing viruses					
Hepadnaviridae	Hepatitis B virus	Circular DS DNA with SS portions	3.2	Yes	I
Parvoviridae	Human parvovirus B-19	SS (+) or (−)DNA	5	No	I
Papoviridae	Human papillomavirus	Circular DS DNA	5–8	No	I
Adenoviridae	Adenovirus	Linear DS DNA	36–38	No	I
Herpesviridae	Herpes simplex virus	Linear DS DNA	120–240	Yes	I
Poxviridae	Vaccinia virus	Linear DS DNA with covalently closed ends	130–380	Yes	Complex

*Influenza C virus: seven segments.
†Reovirus, mammalian reovirus and orbivirus: 10 segments; rotavirus: 11 segments; Colorado tick fever virus: 12 segments.
Abbreviations: DS, Double stranded; H, helical; I, icosahedral; SS, single stranded; Unk, unknown; (+), message sense; (−), complement of message sense.
Data from Murphy and Kingsbury.[40]

From ***Principles and Practice of Infectious Diseases***

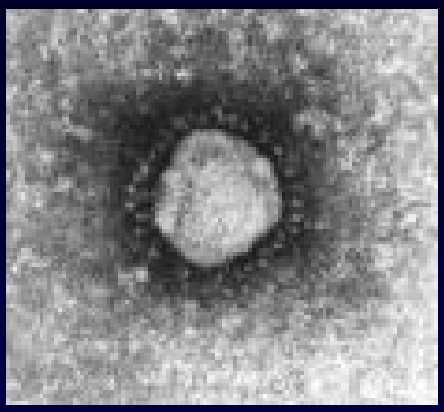
Viruses: Basic Concepts

- 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

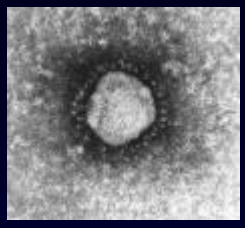


Family: Coronaviridae
(+) SS RNA, enveloped, helical

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**

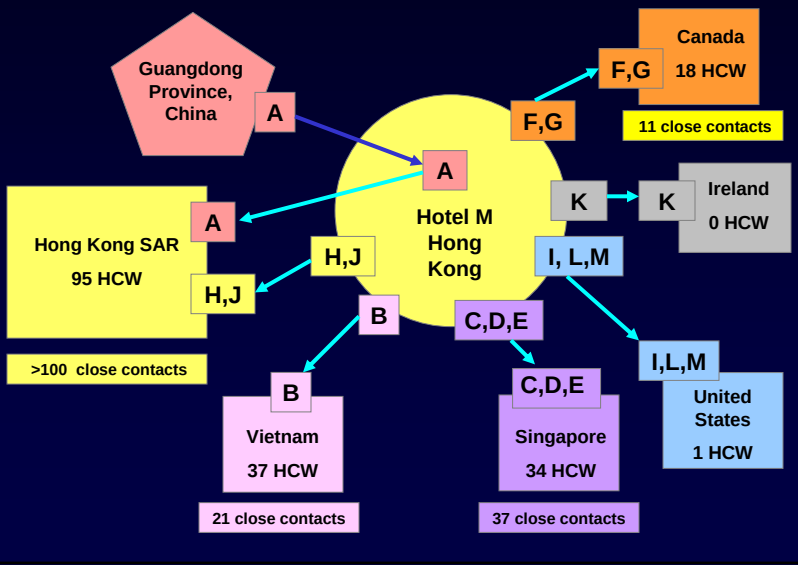
Coronavirus



- 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

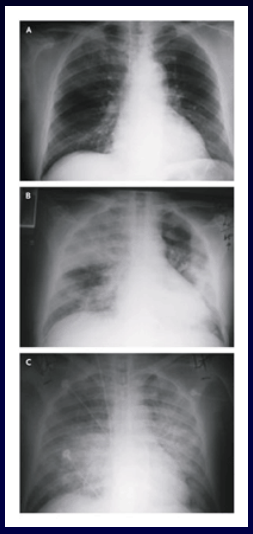
SARS: Clinical Description

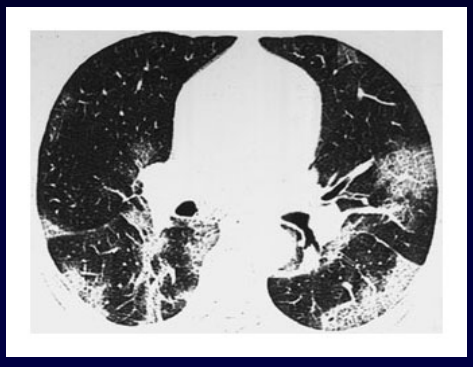
- Incubation period 2 – 7 days
  - Maybe as long as 10 days
- Illness begins with prodrome of fever
  - Chills, headache, malaise, myalgia, diarrhea may also be present
- Next phase: dry cough and/or shortness of breath
- In 10-20% disease may be rapidly progressive and require mechanical ventilation
- 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

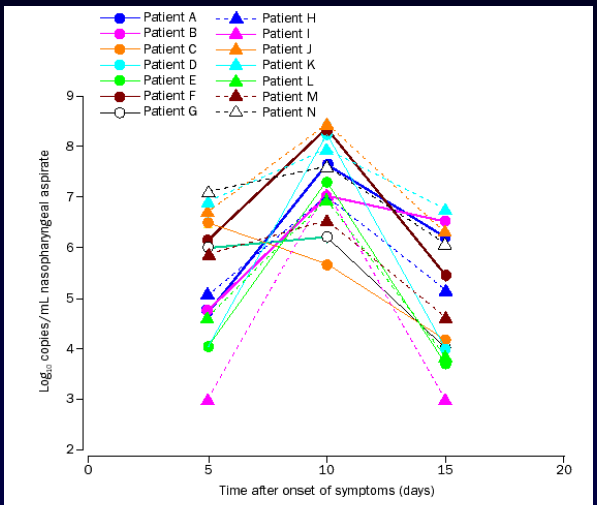
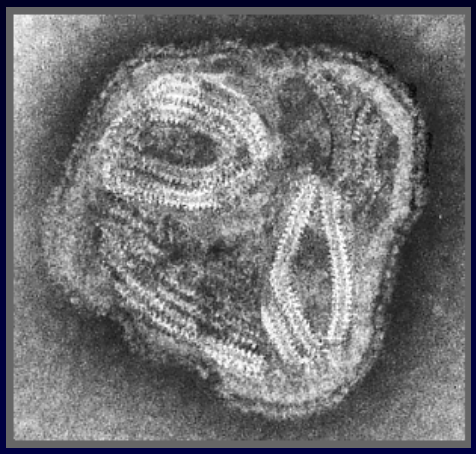


Figure 4: **Sequential quantitative RT-PCR for SARS-associated coronavirus in nasopharyngeal aspirates of 14 SARS patients**

Peiris et al: Lancet, May 24, 2003

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

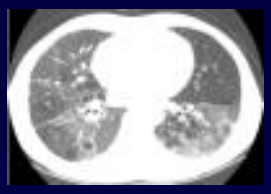
- Complications
  - Pneumonia (giant cell)
  - Encephalitis
  - Subacute sclerosing panencephalitis (SSPE)
    - » Rare in vaccine era, but seen years after measles acquired at an early age (<2)
      - High titers of anti-measles Ab
  - Ocular
  - Atypical measles
    - » Seen in persons exposed to 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

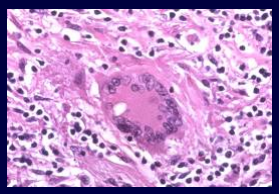
Measles

Rash

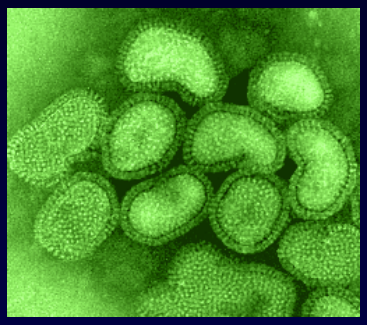
Koplik spots

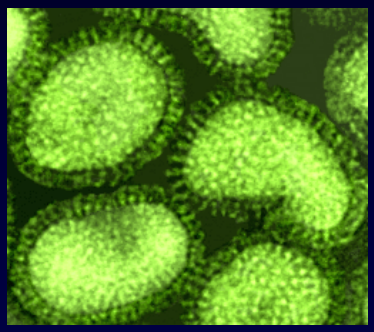
Giant cell pneumonia





Influenza Virus





Family: Orthomyxoviridae
(-) SS RNA segmented, enveloped, helical

Ebola Virus

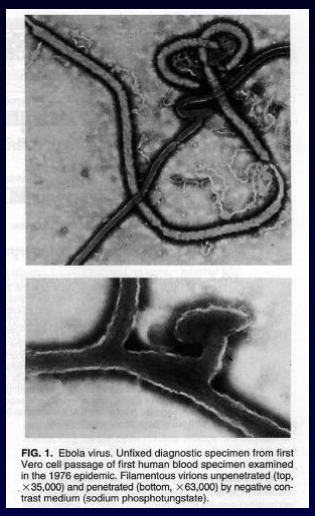
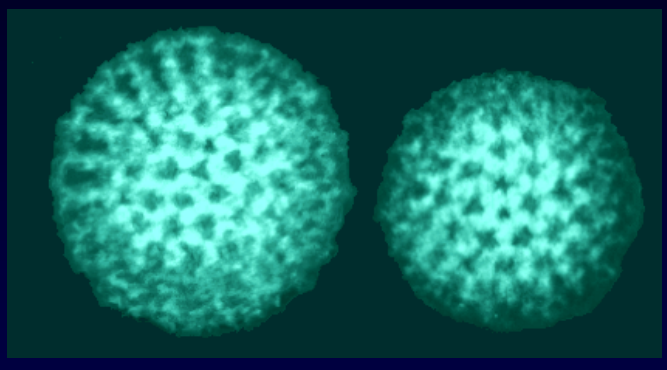


FIG. 1. Ebola virus. Unfixed diagnostic specimen from first Vero cell passage of first human blood specimen examined in the 1976 epidemic. Filamentous virions unpenetrated (top, ×35,000) and penetrated (bottom, ×63,000) by negative contrast medium (sodium phosphotungstate).

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

Rotavirus



Double Capsid **Inner Capsid**

Family: Reoviridae
DS RNA segmented, nonenveloped, icosahedral

Retroviruses

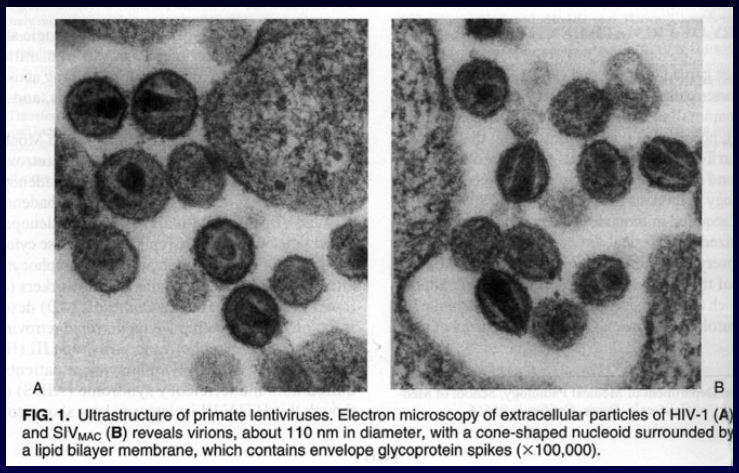
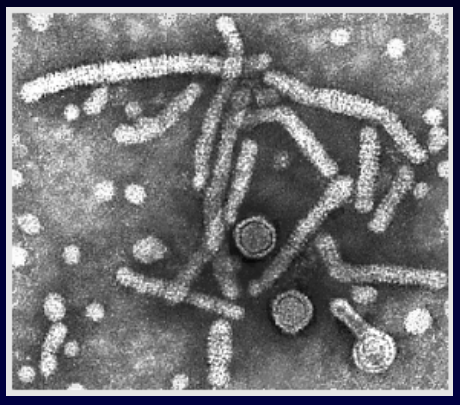


FIG. 1. Ultrastructure of primate lentiviruses. Electron microscopy of extracellular particles of HIV-1 (**A**) and SIV_{MAC} (**B**) reveals virions, about 110 nm in diameter, with a cone-shaped nucleoid surrounded by a lipid bilayer membrane, which contains envelope glycoprotein spikes (×100,000).

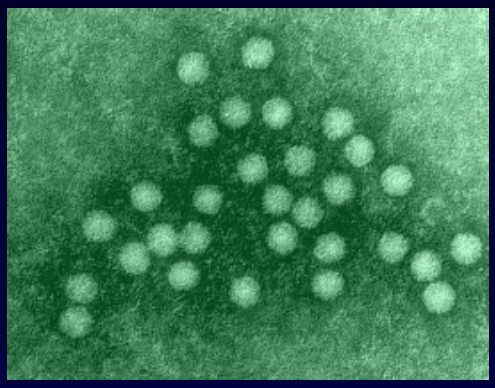
Family: Retroviridae
2 identical (+) RNA strands, enveloped, icosahedral capsid, helical nucleoprotein

Hepatitis B Virus



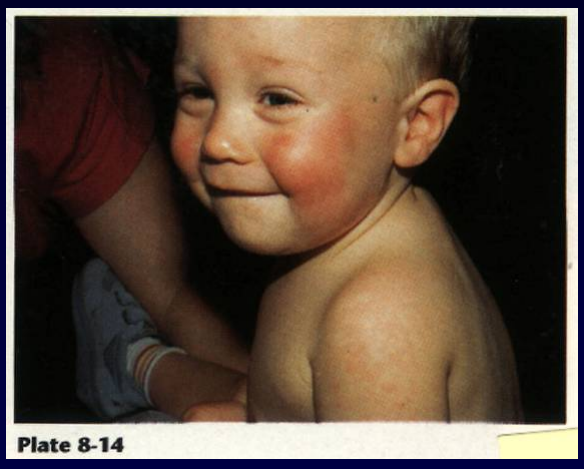
Family: Hepadnaviridae
Circular DS DNA with SS portions, enveloped, icosahedral

Parvovirus



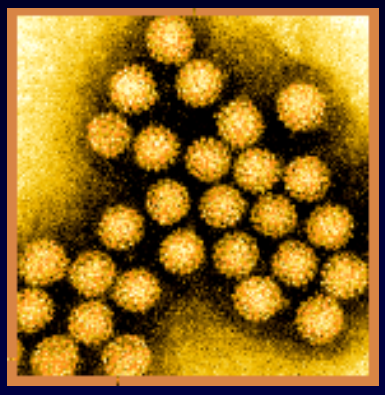
Family: Parvoviridae
SS DNA, nonenveloped, icosahedral

B19 Parvovirus: Erythema Infectiosum



From *Clinical Virology*

Papillomavirus



Family: Papovaviridae
Circular DS DNA, nonenveloped, icosahedral

Cutaneous Wart

From *Clinical Virology*

Cervical Wart

Plate 27-8

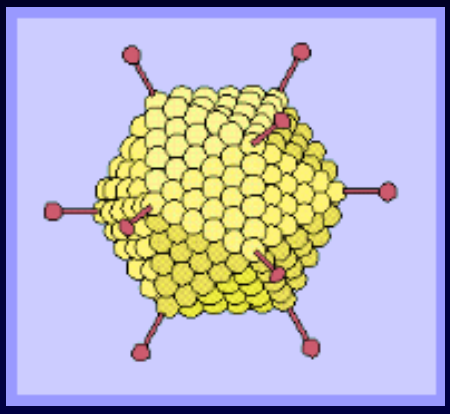
From *Clinical Virology*

Genital Warts

Plate 8-1

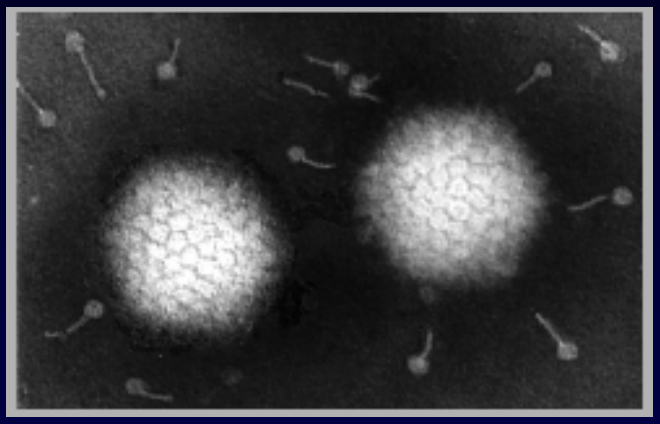
From *Clinical Virology*

Adenovirus



Family: Adenoviridae
Linear DS DNA, nonenveloped, icosahedral

Adenovirus



Family: Adenoviridae
Linear DS DNA, nonenveloped, icosahedral

Adenovirus Conjunctivitis

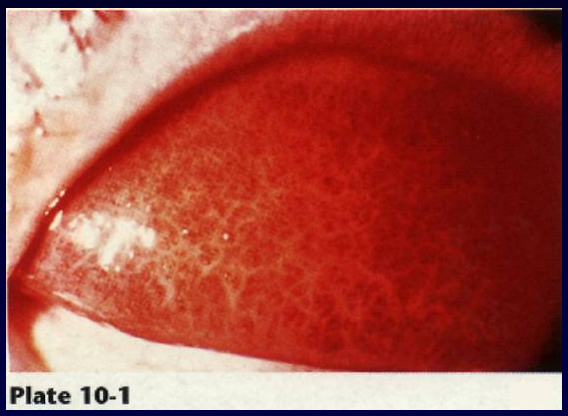


Plate 10-1

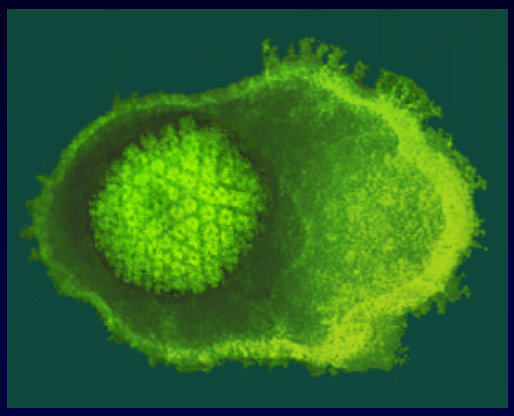
From *Clinical Virology*

Adenovirus Tonsillitis

Plate 25-1

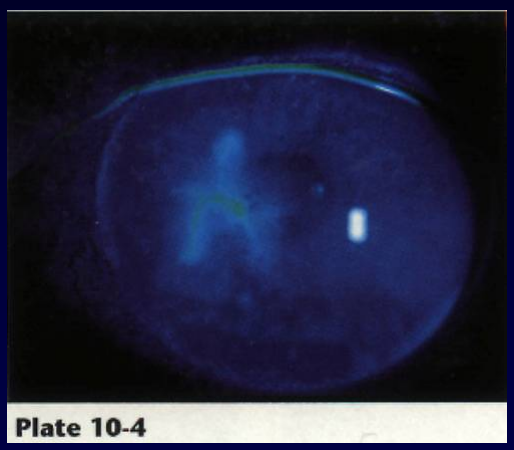
From *Clinical Virology*

Herpesvirus



Family: Herpesviridae
Linear DS DNA, enveloped, icosahedral

Herpes Simplex Virus Keratitis



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Cytomegalovirus Retinitis

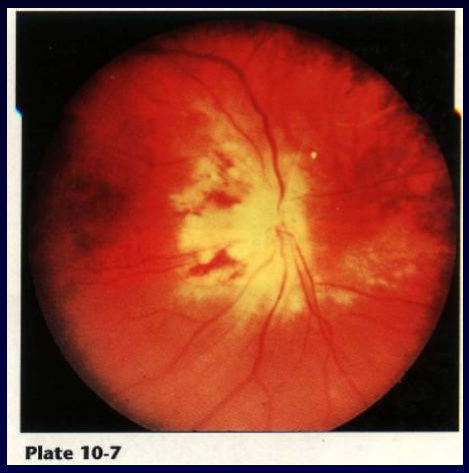
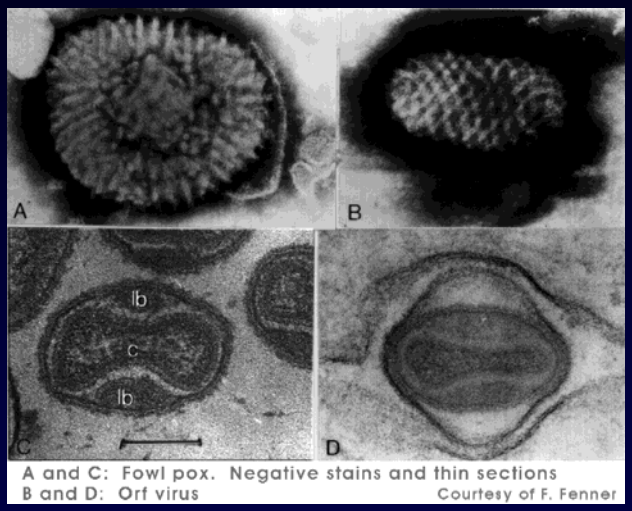


Plate 10-7

From *Clinical Virology*

Poxvirus



A and C: Fowl pox. Negative stains and thin sections
B and D: Orf virus
Courtesy of F. Fenner

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