**Introduction to Virology I: Viral Structure and Function**

I. Background/Discovery

The concept behind modern virology can be traced back to Adolf Mayer, Dimitri Ivanofsky and Martinus Beijerinck who, independently in the late 1880’s, discovered what was later to be called tobacco mosaic virus (TMV). Their discoveries led to the descriptions of filterable agents, too small to be seen with the light microscope, that could be grown in living cells and cause disease. The first filterable agent from animals, foot and mouth disease virus, was described by Loeffler and Frosch in 1898 and the first human filterable agent discovered was yellow fever virus, discovered by Walter Reed in 1901. The term ‘virus’ derives from the Latin for slimy liquid or poison and was gradually introduced during this period to replace the term ‘filterable agents’.

The first virus to be visualized by x-ray crystallography and electron microscopy was TMV, reported in 1941 and 1939, respectively. These advances introduced the notion that viruses were structurally composed of repeating subunits.

Frederick Twort and Felix d’Herelle, working independently, are credited with the discovery of viruses which could infect and lyse bacteria in 1915. D’Herelle introduced the term ‘bacteriophages’ for these agents and also described the concepts of virus adsorption to its target, cell lysis and release of infectious particles. Over the next 35-40 years, work with phages led to numerous discoveries including how the introduction of DNA into a target cell could reproduce itself and the regulation of cellular macromolecular synthesis directed by viruses. In essence, the field of molecular biology was opened up during this period.

Advances in animal virology were noted throughout the 20th century but the major breakthrough came through the development of tissue culture systems that led, for example, to the isolation of poliovirus by Enders et al. in 1949. This markedly facilitated detailed study of this agent and, most importantly, the development of poliovirus vaccines. The ensuing 60 years have seen diagnostic virology mature as a field with the discovery of new agents and diseases and the parallel determination of the importance of viruses in our understanding of molecular biology and cancer.

II. Definitions

A. **Virus particle or virion.** An infectious agent composed of nucleic acid (RNA or DNA), a protein shell (capsid) and, in some cases, a lipid envelope. Virions have full capacity for replication when a susceptible target cell is encountered.

   1. **Capsid and capsomeres.** The protein coat that surrounds the viral nucleic acid. This is composed of repeating protein
subunits called capsomeres. Generally, capsids have either helical or icosahedral symmetry.

2. **Nucleocapsid.** The complete protein-nucleic acid complex.

**B. Satellite or Defective Viruses.** Viruses which require a second virus (helper virus) for replication. Hepatitis delta virus is the major human pathogen example. It requires the presence of hepatitis B virus to complete its replication cycle.

**C. Viroids.** Viroids are the smallest known autonomously replicating molecules. They consist of single-stranded, circular RNA, 240-375 residues in length and are plant pathogens.

**D. Prions.** Prions are not viruses but are often discussed within this microbiologic category. Prions are infectious protein molecules that contain no definable nucleic acid and are responsible for the transmissible and familial spongiform encephalopathies: Creutzfeldt-Jakob disease, kuru, fatal familial insomnia, Gerstmann-Straussler-Scheinker syndrome, and bovine spongiform encephalopathy (“mad cow disease”). The pathogenic prion protein, PrPSc, is formed from a normal human protein, PrPC, through post-translational processing.

**III. Classification**

Viral classification has been confusing and oft-changing over the years. In the past, viruses were often classified by host, target organ or vector and these are still used vernacularly (e.g., the hepatitis viruses). Modern classification is based on the following three characteristics:

**A.** Type of viral nucleic acid (RNA or DNA, single-stranded or double-stranded) and its replication strategy.

**B.** Capsid symmetry (icosahedral or helical).
Figure 119-4, p 1540 from Mandell.

C. Presence or absence of lipid envelope.
IV. Pathogenesis of Viral Diseases

A. As with other infectious agents which cause human disease, the outcome of the interaction of a particular virus with the human host is dependent on both pathogen and host factors. Viral strains within a genus may have differential cell tropisms, replication capacities and cytopathogenic effects. As an example, strains of HIV may preferentially target monocyte/macrophages or T-lymphocytes, may use
different co-receptors (e.g., the chemokine receptors, CCR5 or CXCR4) on the cell surface, may replicate to different levels and may induce different degrees of cell killing. These traits have direct clinical correlates for HIV infected persons with respect to the rates of CD4 cell decline and progression to clinical AIDS. On the host side, the nature of the exposure and the host immune status are probably the two most important determinants of outcome. Thus, the key elements of the virus-host interaction are:

1. Viral strain.
2. Inoculum size.
3. Route of exposure.
4. Susceptibility of host (i.e., is there pre-existent immunity from past exposure or vaccination?).
5. Immune status and age of host.

The net result of this interaction may be:

1. No infection.
2. Abortive infection with limited viral replication.
3. Asymptomatic infection.
4. Symptomatic infection.
5. Depending upon the agent and the immune status of the host, persistent/latent or self-limited infection.

B. Pathogenetic Steps in Human Infection

A generalized schema of viral infection leading to disease in the human host is as follows:

1. Depending upon the agent, the virus enters through the skin, mucous membranes, respiratory tract, gastrointestinal tract, via a transfusion or transplanted organ or via maternal-fetal transmission.
2. There is local replication at the site of the inoculation. Certain agents exhibit pathology at the skin or mucous membrane surface – e.g., herpes simplex virus, human papillomavirus.
3. For some neurotropic viruses there may be spread along peripheral nerve routes to ganglia (e.g., herpes simplex virus) or the central nervous system (e.g., rabies virus). For other neurotropic agents, the central nervous system is seeded following viremia.
4. For many agents, there is replication in regional lymph nodes with subsequent viremia and spread to target organs. Some viruses travel in the bloodstream free in plasma (e.g., picornaviruses); others are cell associated (e.g., cytomegalovirus).
5. Replication in target organs may lead to local damage and further rounds of viremia.
6. Non-specific and specific host immune responses come into play to try to control and downregulate the viral replicative process.

C. Immune Responses to Viral Infections

1. Innate (non-specific) immunity refers to those elements of the immune system that can clear virus or virus infected cells immediately upon or shortly after viral exposure and which are not dependent upon immunologic memory. Non-specific immunity may include:

   a. Phagocytic cells (neutrophils and monocyte/macrophages).
   b. Cytokines (e.g., interferons) and chemokines.
   c. Natural killer cells.
   d. Poorly defined antiviral factors that may exist in blood or body fluids.

2. Adaptive (specific) immunity refers to antigen specific B and T cell responses that lead to the development of antibodies, cytotoxic T cells and antibody dependent cellular cytotoxicity.

3. In some instances, an intense immunologic reaction to a viral agent can result in immunopathology and a serious clinical syndrome. A prime example is dengue hemorrhagic fever which is likely due to antibody dependent enhancement and T cell activation on re-exposure to dengue virus.

D. Mechanisms of Viral Persistence

1. Viruses may cause chronic, persistent infection with continuous viral replication in the face of an immune response. Examples include HIV, hepatitis B virus and hepatitis C virus. Some viruses may demonstrate persistent infection in immune compromised hosts. These include the herpesviruses, human papillomavirus and rubella virus, among others.

2. Some viruses are able to cause latent infection. Latency is characterized by a quiescent or minimally transcriptionally active viral genome with periods of reactivation. Latent viruses include the herpesviruses (cytomegalovirus, Epstein-Barr virus, herpes simplex virus, varicella-zoster virus), human papillomavirus, human retroviruses. Recurrent herpes labialis or genital herpes due to HSV or herpes zoster due to varicella zoster virus are classic examples of latency and reactivation. Viruses which exhibit latency may also exhibit chronic, persistent replication in the setting of immune compromise of the host.
3. Mechanisms of persistence of viruses which produce chronic infections include antigenic variation to escape antibody or cytotoxic T cell responses, downregulation of class I major histocompatibility antigens resulting in diminished recognition by cytotoxic T cells and modulation of apoptosis. Viruses which establish latent infection escape recognition by the immune system through decreased viral antigen expression and presentation.

4. Sites of persistence include the nervous system (herpes simplex virus, varicella zoster virus, measles virus, poliovirus, JC virus), the liver (hepatitis B virus, hepatitis C virus), and leukocytes (HIV, cytomegalovirus, Epstein-Barr virus).

E. Oncogenesis

Several viruses are associated with human cancers. These include: 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; and human papillomavirus with cervical cancer and anogenital carcinoma. Mechanisms of oncogenesis can include transformation (Epstein-Barr virus and herpesvirus 8) and binding of tumor suppressor proteins (human papillomavirus), among others.

V. Diagnosis of Viral Infections

A. The diagnosis of viral infections relies first on the recognition of a distinct clinical syndrome (e.g., herpes zoster infection) or a consideration of the viral infection in the differential diagnosis of a presenting syndrome (e.g., aseptic meningitis).

B. The second consideration is the knowledge of the appropriate specimens to send to the laboratory (blood, body fluids, lesion scraping, tissue) to diagnose a particular infection. One general point to remember is that the isolation of viruses relies on the use of proper viral transport medium and quick delivery to the laboratory.

C. A variety of methods exist to diagnose viral infections with the recent trend being toward molecular diagnostics. These methods include:

1. Isolation of virus in tissue culture, animals, embryonated eggs. Most diagnostic laboratories only use tissue culture for virus isolation. A specific cytopathic effect or induction of a characteristic function (e.g., hemagglutination) can indicate the growth of viruses in tissue culture. This can be confirmed with virus specific antisera applied to the tissue monolayer to neutralize the cytopathic effect or the hemagglutination reaction.
2. Antigen detection in body fluids (e.g., respiratory tract for respiratory viruses) or blood (e.g., cytomegalovirus) or lesion scrapings (e.g., for herpes simplex virus or varicella-zoster virus) with specific immune sera linked to fluorescence or enzyme immunoassay detection.

3. PCR amplification and/or nucleic acid probes to detect viral nucleic acid in body fluids or tissues.

4. Antibody detection. IgM antibody detection can assist with acute diagnosis. Four-fold rises in IgG specific antibody or conversion from seronegative status to seropositive status can secure a diagnosis but this may not be helpful in the acute setting.

5. Examination of tissue samples by light microscopy for viral induced cytopathology and antigen detection by immunohistochemical staining.

6. Examination of body fluids or tissues by electron microscopy. This is not an efficient method and is dependent upon sufficient numbers of virions being present to permit detection.

VI. Prevention and Therapy

A. Vaccines for the prevention of life threatening viral infections are one of the most significant advances in human health. The eradication of smallpox is the hallmark example of the effectiveness of a viral vaccine. Effective vaccines exist for polio, mumps, measles, rubella, influenza, hepatitis A, hepatitis B, varicella-zoster, rabies, adenovirus, Japanese B encephalitis and yellow fever.

B. Immune globulin can prevent or ameliorate clinical disease due to certain viral agents. Examples include varicella-zoster immune globulin for exposure in immune compromised hosts, rabies immune globulin (administered with rabies vaccine) following an exposure, cytomegalovirus immune globulin for transplant recipients, respiratory syncytial virus immune globulin and immune serum globulin for hepatitis A.

C. Screening of blood for prevention of transmission of HIV, hepatitis B, hepatitis C and in certain transplant situations, cytomegalovirus.

D. Safe sexual practices for the prevention of HIV, hepatitis B and human papillomavirus infections.

E. Advances in specific antiviral therapy over the past 30 years have been marked. Effective therapy exists for herpes simplex virus, varicella-zoster virus, cytomegalovirus, HIV, influenza virus, respiratory syncytial virus, hepatitis B and hepatitis C.