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## **Virus-Cell Interactions and Viral Pathogenesis**

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Knowledge of viral replication and genetics is necessary for understanding the interaction between the virus and the host cell. The interaction at the cellular level and progression of a particular viral infection determines disease pathogenesis and clinical manifestations. The host immune response to the presence of viruses is examined in Chapter 5.

### **Interaction Between Viruses and Host Cells**

The interaction between viruses and their host cells is intimately tied to the replication cycle of the virus. Moreover, the interaction of virus with cellular components and structures during the replication process influences how viruses cause disease. Overall, there are four possible primary effects of viral infection on a host cell. Most infections cause no apparent cellular pathology or morphological alteration; however, replication may cause cytopathology (cell rounding, detachment, syncytium formation, etc.), malignant transformation, or cell lysis (death).

### **Cell Death**

Cell death during viral replication can be caused by a variety of factors. The most likely factor is the inhibition of basal cellular synthesis of biomolecules, such as proteins. During the replication cycle, the virus induces the cellular machinery to manufacture largely viral products rather than those the cell would normally make. As a result, the predominant products synthesized by the cell are viral and the cellular products necessary for the survival of the cell are not present or present in too low a quantity to maintain its viability. In addition to the lack of essential cellular products, this event results in accumulation of viral products (RNA, DNA, proteins) in excess, which can be toxic for the cells.

In the release phase of the replication cycle of some viruses apoptosis of the host cell is stimulated. In other instances, inhibition of the synthesis of cellular macromolecules causes damage to lysosomal membranes and subsequent release of hydrolytic enzymes resulting in cell death.

### **Cellular Effects**

Cytopathic effect (CPE) denotes all morphologic changes in cells resulting from virus infection. Infected cells sometimes have an altered cell membrane; as a result the infected cell membrane is capable of fusing with its neighbor cell. It is thought this altered membrane is the result of the insertion of viral proteins during the replication cycle. The result of fusion is the generation of a multinucleate cell or syncytia. The formation of syncytia is characteristic for several enveloped viruses, such as herpesviruses and paramyxoviruses. The altered cell membrane also is altered with regard to its permeability, allowing influxes of various ions, toxins, antibiotics, etc. These multinucleate cells are large and are sometimes called "multinucleate giant cells".

Another aspect of CPE is the disruption of the cytoskeleton, leading to a "rounded up" appearance of the infected cell. The cell in this case will either lyse or form syncytia. CPE occurrence in clinical specimens can indicate viral infection and CPE is used as the basis for the plaque assay used in viral enumeration (see Chapter 2).

Infection of cells with some viruses (e.g., poxviruses and rabies virus) is characterized by the formation of cytoplasmic inclusion bodies. Inclusion bodies are discrete areas containing viral proteins or viral particles. They often have a characteristic location and appearance within an infected cell, depending upon the virus.

### **Malignant Transformation**

In this process, viral infection results in host cells that are characterized by altered morphology, growth control, cellular properties, and/or biochemical properties. Malignant transformation and resulting neoplasia may occur when the viral genome (or a portion) is incorporated into the host genome or when viral products are themselves oncogenic. Viruses causing malignant transformation are referred to as tumor viruses. Viruses from different families have been shown to possess the ability to transform host cells. The tumor viruses have no common property (size, shape, chemical composition) other than the development of malignancy in the host cell.

Malignant transformation is often characterized by altered cellular morphology. This includes the loss of their characteristic shape and assumption of a rounded up, refractile appearance as described for CPE. This is the result of the disaggregation of actin filaments and decreased surface adhesion.

Altered cell growth, the hallmark for malignant transformation, is exhibited in viral cells that have lost contact inhibition of growth or movement, have a reduced requirement for serum growth factors, and/or no longer respond to cell cycle signals associated with growth and maturation of the cell (immortality).

Some of the altered cellular properties exhibited by malignantly transformed cells include continual induction of DNA synthesis, chromosomal changes, appearance of new or embryonic surface antigens, and increased agglutination by lectins. Commonly altered biochemical properties of malignantly transformed cells include reduced levels of cyclic AMP. Cyclic AMP is a chemical signal associated with the cell cycle and by keeping the levels reduced the cell continually divides. Also involved is the increased secretion of plasminogen activator (clot formation), fermentation for the production of lactic acid (known as the Warburg effect), loss of fibronectin, and changes in the sugar components of glycoproteins and glycolipids.

### **Oncogenesis**

Although cause-and-effect has been difficult to obtain, a number of DNA and RNA viruses have been associated with neoplastic transformation. Viruses implicated in oncogenesis either carry a copy of a gene associated with cell growth and proliferation or alter expression of the host cell's copy of the gene. Effected genes include those that stimulate and those that inhibit cell growth.

Viral genes that transform infected cells are known as oncogenes (*v-*onc** genes), which stimulate uncontrolled cell growth and proliferation. The discovery of oncogenes led to the finding that all cells contain analogous genes, called proto-oncogenes (*c-*onc** genes), which are normally quiescent in cells as they are active at some point in development. Proto-oncogenes include cellular products such as growth factors, transcription factors, and growth factor receptors.

DNA viruses associated with oncogenesis include the Marek's disease virus (Herpesviridae) and the bovine, equine, and canine oral papillomaviruses (Papillomaviridae). These viruses are typically circular episomic (independent of the host chromosome, rather than integrated) nucleic acids. The oncogenes (*v-*onc**) encode proteins associated with the replication cycle of the virus.

RNA viruses associated with oncogenesis include members of the family Retroviridae (e.g., avian leukosis virus and feline leukemia virus). These viruses integrate their genomes (or a copy of the genome) into the host chromosome; referred to as proviruses or proviral DNA. Viral integration is mediated by the terminal ends of the genome, known as LTRs (long terminal repeats). LTRs contain promoter/enhancer regions, in addition to sequences involved with integration of the provirus into the host genome. Retroviruses can cause oncogeneses by encoding oncogenes themselves or by altering the expression of cellular oncogenes or proto-oncogenes through insertion of their genomes into the host chromosome close to these genes.

### **No Morphological or Functional Changes**

In some instances, infection with viral production can occur with no discernable change in the host cell. This is referred to as an endosymbiotic infection. This is probably dependent upon the replication needs of the virus. Most likely the virus requires cellular processes to be active in order for viral replication to take place and thus does not alter the features of the cell.

### **Pathogenesis of Viral Infections**

Pathogenesis is defined as the origination and development of a disease. Viral infections can be acute, chronic, latent or persistent. The first step in the disease process is exposure.

### **Exposure and Transmission**

Exposure may occur by direct contact with an infected animal, by indirect contact with secretions / excretions from an infected animal, or by mechanical or biological vectors. Transmission of virus from mother to offspring (transplacental, perinatal, colostrum) is called vertical transmission. Transmission via other than mother to offspring is horizontal transmission.

Activation of latent, nonreplicating virus can occur within an individual with no acquisition of the agent from an exogenous source.

### **Portal of Entry**

Viruses enter the host through the respiratory tract (aerosolized droplets), the alimentary tract (oral-fecal contamination), the genitourinary tract (breeding, artificial insemination), the conjunctivae (aerosolized droplets), and through breaches of

the skin (abrasions, needles, insect bites, etc.). Whether or not infection ensues following entry depends upon the ability of the virus to encounter and initiate infection in susceptible cells. The susceptibility of cells to a given virus depends largely on their surface receptors, which allow for attachment and subsequent penetration of the virus.

### **Localized and Disseminated Infections**

Following infection, the virus replicates at or near the site of viral entry (primary replication). Some viruses remain confined to this initial site of replication and produce localized infections. An example is the common cold and similar infections in domestic animals caused by rhinovirus.

Other viruses cause disseminated (systemic) infections by spreading to additional organs via the bloodstream, lymphatics or nerves. The initial spread of virus to other organs by the blood stream is referred to as primary viremia. Viremia can be either by virus free in the plasma or by virus associated with blood cells. After multiplication in these organs, there may be a secondary viremia with spread to target organs.

A good example of a virus causing a systemic infection is porcine teschovirus 1 (genus Teschovirus). The virus is transmitted in a fecal-oral fashion. It initially replicates in the cells of the tonsils, migrates to the intestines and mesenteric lymph nodes. From the mesenteric lymph nodes, the virus enters the central nervous system. Once in the central nervous system, the neurological symptoms of: ataxia, tremors, loss of coordination, stiffening of the limbs, convulsions, paralysis, and coma are observed. The preference of a particular virus for a specific tissue or cell type is known as tropism.

### **Mechanisms of Viral Infections**

Virus replication occurs in target organs causing cell damage. The number of cells infected and/or the extent of damage may result in tissue/organ dysfunction and in clinical manifestation of disease. The interval between initial infection and the appearances of clinical signs is the incubation period. Incubation periods are short in diseases in which the virus grows rapidly at the site of entry (e.g., influenza) and longer if infections are generalized (e.g., canine distemper). Some viruses infect animals but cause no overt signs of illness. Such infections are termed subclinical (asymptomatic or unapparent). There are numerous factors that may influence the outcome of viral infections. These include preexisting immunity, genetics of the animal, age of the animal, and stress related factors such as nutritional status, housing, etc.

The mechanisms by which viruses cause disease are complex. Disease may result from direct effects of the virus on host cells, such as cell death, CPE, and malignant transformation. Alternatively, disease results from indirect effects caused by the immunologic and physiologic responses of the host.

An example of indirect physiologic response is infection with rotavirus, which causes diarrhea in young animals and humans. Diarrhea may be caused by rotavirus-infected erythrocytes that are stimulated to produce cytokines, exciting enteric neurons, and inducing the secretion of excess fluids and electrolytes into the large intestine.

An example of immunologic response mediating disease pathogenesis occurs with Borna disease in horses. The virus spreads from the CNS to peripheral nerves within axons. The host responds to the presence of the virus-infected neurons by inducing a cell-mediated immune response. Macrophages, neutrophils, and specific cytotoxic T lymphocytes are activated to kill bornavirus-infected neurons. The result is chronic inflammation in the CNS that corresponds with the neurological signs associated with the disease.

Two very important terms used in the discussion of microbial diseases are pathogenicity and virulence. Pathogenicity denotes the ability of a virus or other microbial/parasitic agent to cause disease. Virulence is the degree of pathogenicity. An avirulent virus is one lacking the capacity to cause disease. An attenuated virus is one whose capacity to cause disease has been weakened frequently by multiple passages in cell cultures, embryonated eggs or animals.

### **Virus Shedding**

Virus shedding is the mechanism of excretion of the progeny virions to spread to a new host, thus maintaining the virus in a population of hosts. Viruses are typically shed via body openings or surfaces. For localized infections, virus is typically shed via the portal of entry. In disseminated infections, virus may be shed by a variety of routes.

Not all viruses are shed from their hosts. These include viruses that replicate in sites such as the nervous system, as in viral encephalitis, and dead-end hosts.

### **Evasion of Host Defenses**

In an effort to ward off the infection, the host initiates an inflammatory response. Principal components of this response include interferons, cytotoxic T lymphocytes, antibody producing B-lymphocytes, a variety of effector molecules, and complement. These various components work in concert and augment one another in an attempt to rid the host of the infecting virus. In this effort to rid itself of the infecting virus, the inflammatory response causes many of the clinical signs and lesions associated with viral infections. The host immune response is discussed in greater detail in Chapter 5.

Interferons ( $\alpha$  and  $\beta$ ) are produced by virus-infected cells. They act to stop further virus replication in the infected and neighboring cells. Interferons also enhance antigen expression on infected cells, thereby making them more recognizable to cytotoxic T cells. Some viruses (e.g., adenovirus) produce RNAs that block the phosphorylation of an initiation factor, that reducing the ability of interferon block viral replication.

Cytotoxic T cells kill viral infected cells by releasing perforins, which create pores in the virus-infected cell. Granzymes are then released into the virus-infected cell, which degrade the cell components. Lastly, cytotoxic T cells stimulate

apoptosis of the host cell.

Some viruses reduce the expression of MHC class I antigens on the surface of the host cell (e.g., cytomegalovirus, bovine herpesvirus type I, adenoviruses). As cytotoxic T cells cannot detect viral antigens that are not complexed with MHC class I antigens, virus-infected cells cannot be destroyed in this manner, allowing "survival" of the virus within the host.

However, cells with no or insufficient MHC class I antigen on their surface are recognized by natural killer cells, which kill the cell in a manner similar to that described for cytotoxic T cells.

Antibody producing B-lymphocytes secrete specific antibodies to neutralize the infectious virions when the cell liberates them. Antigen-antibody complexes in turn can activate the complement system.

Complement aids in stimulating inflammation and the effective neutralization of virus and in the destruction of viral infected cells.

The various effector molecules (cytokines) that are produced by the cells of the immune system have many roles, including the induction of fever and the attraction of other inflammatory cells, (e.g., neutrophils and macrophages) to the injured site.

Some viruses possess receptors for a variety of cytokines (e.g., vaccinia virus has receptors for interleukin-1, which stimulates fever production). When immune cells release the cytokine, it is bound to the virus. This, in turn, reduces the amount of the cytokines available to modulate immune responses. This enhances the "survival" of the virus within the host.

An alternate mechanism to evade the immune response is to have many antigenic types (serotypes). An immune response to one serotype does not guarantee protection from another serotype of the same virus. For example, there are over 100 serotypes of rhinovirus and 24 serotypes of bluetongue virus.

### **Persistent Viral Infections**

Some viruses have the ability to abrogate the inflammatory response and cause persistent infections. They accomplish this in a number of ways, including the destruction of T lymphocytes causing immunosuppression, the avoidance of immunologic surveillance by altering antigen expression, and by the inhibition of interferon production.

There are three clinically important types of persistent infections:

#### Chronic-carrier infections

These are organisms that continually produce and shed large quantities of virus for extended periods of time. As a result they continually spread the virus to others. Some chronic-carriers are asymptomatic or exhibit disease with very mild symptoms. Examples include infections with equine arteritis virus, feline panleukopenia virus, and avian polyoma virus.

#### Latent infections

A special type of persistent infection is one in which the virus is maintained in the host in a "non-productive" state.

Herpesviruses are notorious for causing latent infections. The viral genome is maintained in neurons in a closed circular form, and is periodically reactivated (often during stressful conditions) resulting in a productive infection and viral shedding.

Latent infections also occur with retroviruses in which the proviral DNA is incorporated into the host cell genome. Cell transformation and malignancy may result if the integrated transcript causes a disruption of normal cellular control processes.

#### Slow Virus Infections

This refers to those viral infections in which there is a prolonged period between initial infection and onset of disease. In this case, viral growth is not slow, but rather the incubation and progression of disease are extended. An example is the disease subacute sclerosing panencephalitis, which develops several years following measles virus (paramyxovirus) infection. "Old dog encephalitis" due to recrudescence of distemper would appear to be an analogous condition.

### **Glossary**

Antigen: A substance, usually external to the body but occasionally within the body, which the immune system recognizes as foreign or non-self. When thus recognized it elicits a specific antibody which reacts with it.

Apoptosis: A form of programmed cell death characterized by the fragmentation of nuclear DNA.

Cytokines: A diverse group of small (< 30 kilodalton), soluble proteins produced by leukocytes that mediate a variety of immune functions.

Cytotoxic T lymphocytes: Cells that recognize foreign antigens imbedded in MHC class I molecules. They are only effective in killing cells containing foreign antigen.

Endosymbiotic: Form of symbiosis where one organism lives within another.

Granzymes: A collection of serine proteases; they pass into the target cell via the transmembrane channels created by perforin, where they interact with the various intracellular pathways that trigger apoptosis and DNA degradation.

Interferons: Comprised of three different proteins designated alpha, beta, and gamma. All have a non-specific action against viruses, but alpha and beta are the more potent.

Interleukins: A group of cytokines secreted by effector cells of the immune system that effect responses by other immune cells.

Lectins: Plant glycoproteins that bind specifically to certain sugars, some of which occur on the surface of cells.

Macrophages: The main phagocyte of tissues, organs and such serous membranes as the pleura and peritoneum.

MHC class I antigens (major histocompatibility complex): Collection of genes coding for the self-marking proteins or major irreconcilability antigens. These antigens occur on the surface of all body cells and serve to identify them as belonging to the body and not foreign. Some MHC antigens appear on the surface of cells of the immune system. The human MHC region is known as the HLA (human leukocyte antigen) region and is located on chromosome 6.

Natural killer cells (NK cells): Cytotoxic lymphocytes, which comprise approximately 5 to 15% of circulating lymphocytes, lack the phenotypic markers of T and B cells. They have capacity to kill certain tumor cells and virus-infected cells that lack major histocompatibility (MHC) markers on cell surfaces and their mechanism of killing is similar to that of cytotoxic T cells.

Neutrophils: A short-lived phagocytic cell with granules that contain a number of bactericidal compounds; most numerous of the circulating leukocytes constituting approximately 60 to 70% in humans. Microscopically they have an irregularly shaped, multi-lobed nucleus; also called polymorphonuclear leukocytes.

Perforins: A pore-forming protein that requires the presence of calcium in order to polymerize and form transmembrane channels in the plasma membrane of the target cell.

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