

## Viral Pathogenesis

This lecture will define and discuss the basic principles of **viral pathogenesis**, the entire process by which viruses cause **disease**. Viral disease is a sum of the effects on the host of virus replication and of the immune response.

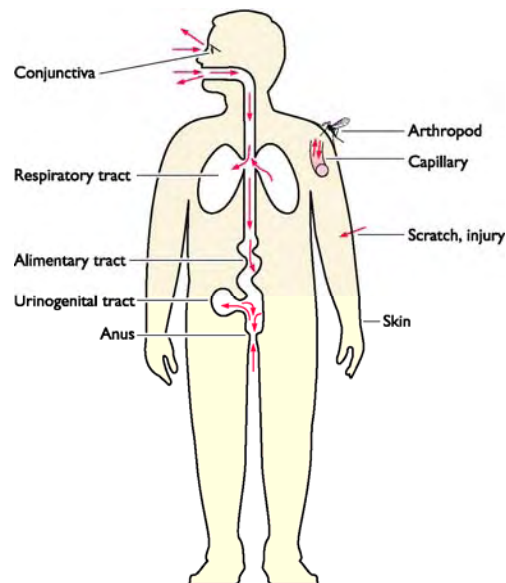
Interest in viral pathogenesis stems from the desire to treat or eliminate viral diseases that affect humans. This goal is achieved in part by identifying the viral and host genes that influence the production of disease. Progress in understanding the molecular basis of viral pathogenesis comes largely from studies of animal models. The mouse has become a particularly fruitful host for studying viral pathogenesis because the genome of this animal can be manipulated readily. In some cases, non-human hosts can be infected with the same viruses that infect humans, but close relatives of human viruses must often be used.

### Viral Entry

Three requirements must be satisfied to ensure successful infection in an individual host:

- Sufficient virus must be available to initiate infection
- Cells at the site of infection must be accessible, susceptible, and permissive for the virus
- Local host anti-viral defense systems must be absent or initially ineffective.

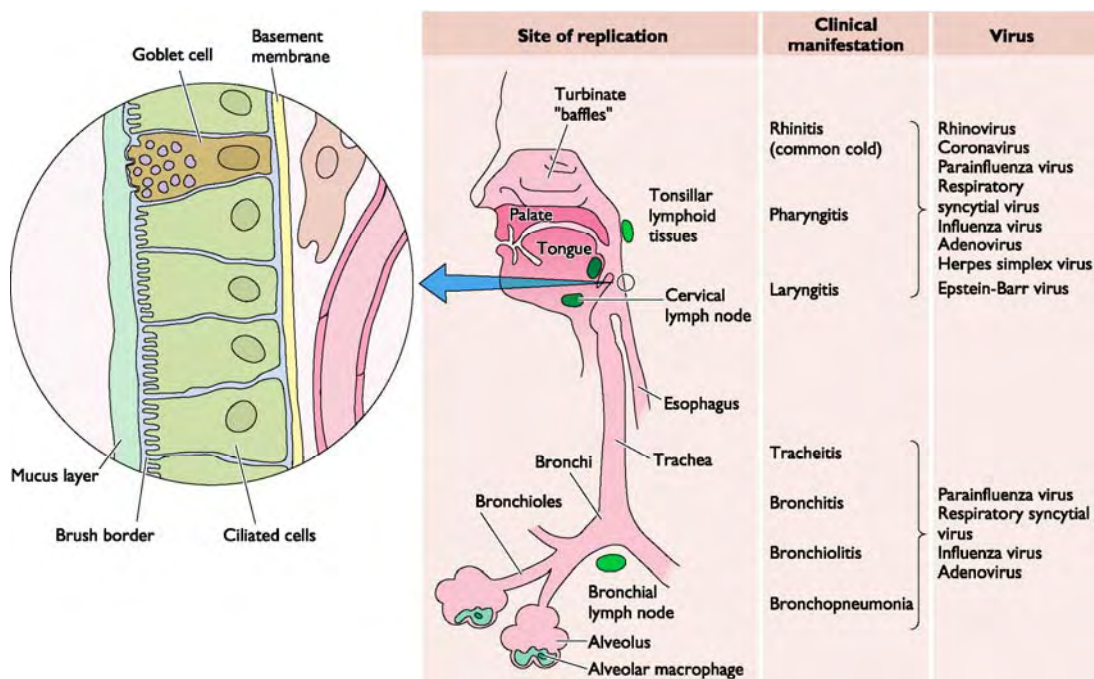
To infect its host, a virus must first enter cells at a body surface. Common sites of entry include the mucosal linings of the respiratory, alimentary, and urogenital tracts, the outer surface of the eye (conjunctival membranes or cornea), and the skin (Fig. 1).



**Figure 1. Sites of viral entry into the host.**

### **Respiratory Tract**

The most common route of viral entry is through the respiratory tract. The combined absorptive area of the human lung is almost 140 m<sup>2</sup>. Humans have a resting ventilation rate of 6 liters of air per minute, which introduces large numbers of foreign particles and aerosolized droplets into the lungs with every breath. Many of these particles and droplets contain viruses. Fortunately, there are numerous host defense mechanisms to block respiratory tract infection. Mechanical barriers play a significant role in anti-viral defense. For example, the tract is lined with a mucociliary blanket consisting of ciliated cells, mucous-secreting goblet cells, and sub-epithelial mucous-secreting glands (Fig. 2). Foreign particles deposited in the nasal cavity or upper respiratory tract are trapped in mucus, carried to the back of the throat, and swallowed. In the lower respiratory tract, particles trapped in mucus are brought up from the lungs to the throat by ciliary action. The lowest portions of the tract, the alveoli, lack cilia or mucus, but macrophages lining the alveoli ingest and destroy particles. Other cellular and humoral immune responses also intervene.



**Figure 2. Sites of viral entry in the respiratory tract.**

Viruses may enter the respiratory tract in the form of aerosolized droplets expelled by an infected individual by coughing or sneezing, or through contact with saliva from an infected individual. Larger virus-containing droplets are deposited in the nose, while smaller droplets find their way into the airways or the alveoli. To infect the respiratory tract successfully, viruses must not be swept away by mucus, neutralized by antibody, or destroyed by alveolar macrophages.

### **Alimentary Tract**

The alimentary tract is a common route of infection and dispersal. Eating, drinking, and some social activities routinely place viruses in the alimentary tract. It is designed to mix, digest, and absorb food, providing a good opportunity for viruses to encounter a susceptible cell and to interact with cells of the circulatory, lymphatic, and immune systems. It is an extremely hostile environment for a virus. The stomach is acidic, the intestine is alkaline, digestive enzymes and bile detergents abound, mucus lines the epithelium, and the luminal surfaces of intestines contain antibodies and phagocytic cells. Viruses that infect by the intestinal route must, at a

minimum, be resistant to extremes of pH, proteases, and bile detergents. Indeed, viruses that lack these features are destroyed when exposed to the alimentary tract, and must infect at other sites.

The hostile environment of the alimentary tract actually facilitates infection by some viruses. For example, reovirus particles are converted by host proteases in the intestinal lumen into infectious subviral particles, the forms that subsequently infect intestinal cells. As might be expected, most enveloped viruses do not initiate infection in the alimentary tract, because viral envelopes are susceptible to dissociation by detergents such as bile salts. Enteric coronaviruses are notable exceptions, but it is not known why these enveloped viruses can withstand the harsh conditions in the alimentary tract.

Nearly the entire intestinal surface is covered with columnar villous epithelial cells with apical surfaces that are densely packed with microvilli (Fig. 3). This brush border, together with a surface coat of glycoproteins and glycolipids, and the overlying mucous layer, is permeable to electrolytes and nutrients, but presents a formidable barrier to microorganisms. Nevertheless, viruses such as enteric adenoviruses and Norwalk virus, a calicivirus, replicate extensively in intestinal epithelial cells. The mechanisms by which they bypass the physical barriers and enter susceptible cells are not well understood. Scattered throughout the intestinal mucosa are lymphoid follicles that are covered on the luminal side with a specialized follicle-associated epithelium consisting mainly of columnar absorptive cells and M cells (membranous epithelial cells). M-cell transcytosis is believed to provide the mechanism by which some enteric viruses gain entry to deeper tissues of the host from the intestinal lumen.

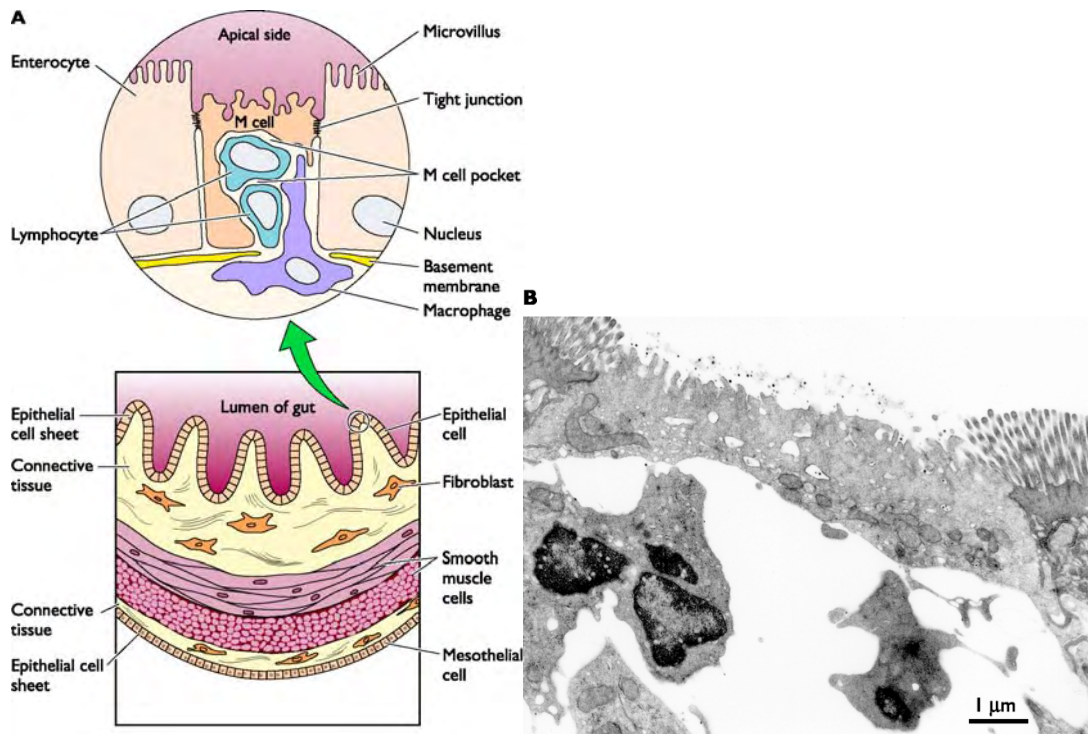


Figure 3. Viral entry in the intestine through M cells.

### ***Urogenital Tract***

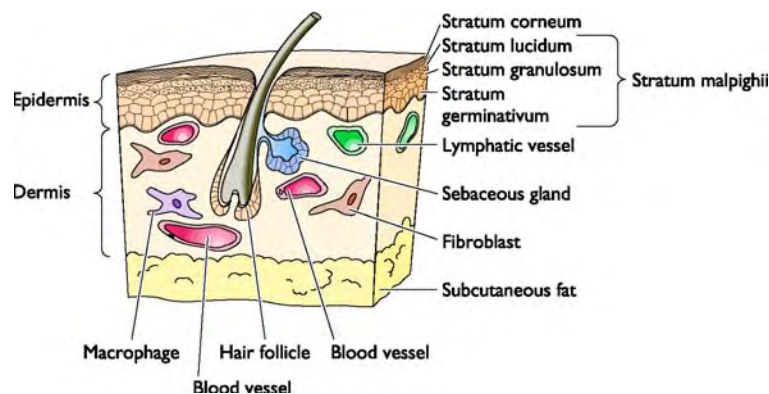
Some viruses enter the urogenital tract as a result of sexual activities. The urogenital tract is well protected by physical barriers, including mucus and low pH (in the case of the vagina). Normal sexual activity can result in minute tears or abrasions in the vaginal epithelium or the urethra, allowing viruses to enter. Some viruses infect the epithelium and produce local lesions (e.g., certain human papillomaviruses, which cause genital warts). Other viruses gain access to cells in the underlying tissues and infect cells of the immune system (e.g., human immunodeficiency virus type 1), or sensory and autonomic neurons (in the case of herpes simplex viruses).

### ***Eyes***

The epithelium covering the exposed part of the sclera and the conjunctivae is the route of entry for several viruses. Every few seconds the eyelid passes over the sclera, bathing it in secretions that wash away foreign particles. There is usually little opportunity for viral infection of the eye, unless it is injured by abrasion. Direct inoculation into the eye may occur during ophthalmologic procedures or from environmental contamination (e.g., improperly sanitized swimming pools). In most cases, replication is localized and results in inflammation of the conjunctiva (conjunctivitis). Systemic spread of the virus from the eye is rare, although it does occur (e.g., paralytic illness after enterovirus 70 conjunctivitis). Herpesviruses can also infect the cornea at the site of a scratch or other injury. This infection may lead to immune destruction of the cornea and blindness.

### ***Skin***

The skin of most animals is an effective barrier against viral infections, as the dead outer layer cannot support viral growth (Fig. 4). Entry through this organ occurs primarily when its integrity is breached by breaks or punctures. Replication is usually limited to the site of entry because the epidermis is devoid of blood or lymphatic vessels that could provide pathways for further spread. Other viruses can gain entry to the vascularized dermis through the bites of arthropod vectors such as mosquitoes, mites, ticks, and sandflies. Even deeper inoculation, into the tissue and muscle below the dermis, can occur by hypodermic needle punctures, body piercing or tattooing, animal bites, or sexual contact when body fluids are mingled through skin abrasions or ulcerations. In contrast to the strictly localized replication of viruses in the epidermis, viruses that initiate infection in dermal or sub-dermal tissues can reach nearby blood vessels, lymphatic tissues, and cells of the nervous system. As a consequence, they may spread to other sites in the body.



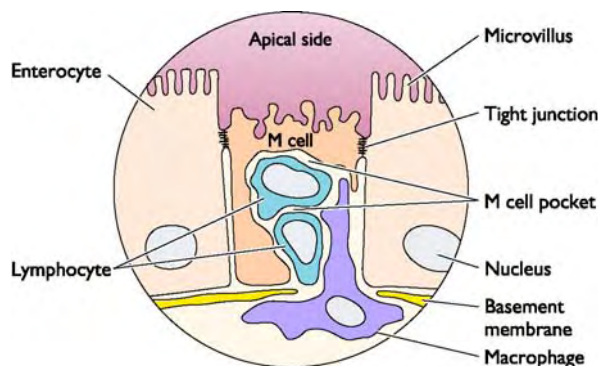
**Figure 4. Diagram of the skin.**

**Table 1. Routes of virus entry into the host**

Location	Virus(es)
<b>Respiratory tract</b>	
Localized upper tract	Rhinovirus; coxsackievirus; coronavirus; arenaviruses; hantavirus; parainfluenza virus types 1–4; respiratory syncytial virus; influenza A and B viruses; human adenovirus types 1–7, 14, 21
Localized lower tract	Respiratory syncytial virus; parainfluenza virus types 1–3; influenza A and B viruses; human adenovirus types 1–7, 14, 21; infectious bovine rhinotracheitis virus (herpesvirus)
Entry via respiratory tract followed by systemic spread	Foot-and-mouth disease virus, rubella virus, arenaviruses, hantavirus, mumps virus, measles virus, varicella-zoster virus, poxviruses
<b>Alimentary tract</b>	
Systemic	Enterovirus, reovirus, adenovirus
Localized	Coronavirus, rotavirus
<b>Urogenital tract</b>	
Systemic	Human immunodeficiency virus type 1, hepatitis B virus, herpes simplex virus
Localized	Papillomavirus
<b>Eyes</b>	
Systemic	Enterovirus 70, herpes simplex virus
<b>Skin</b>	
Arthropod bite	Bunyavirus, flavivirus, poxvirus, reovirus, togavirus
Needle puncture, sexual contact	Hepatitis C and D viruses, cytomegalovirus, Epstein-Barr virus, hepatitis B virus, human immunodeficiency virus, papillomavirus (localized)
Animal bite	Rhabdovirus

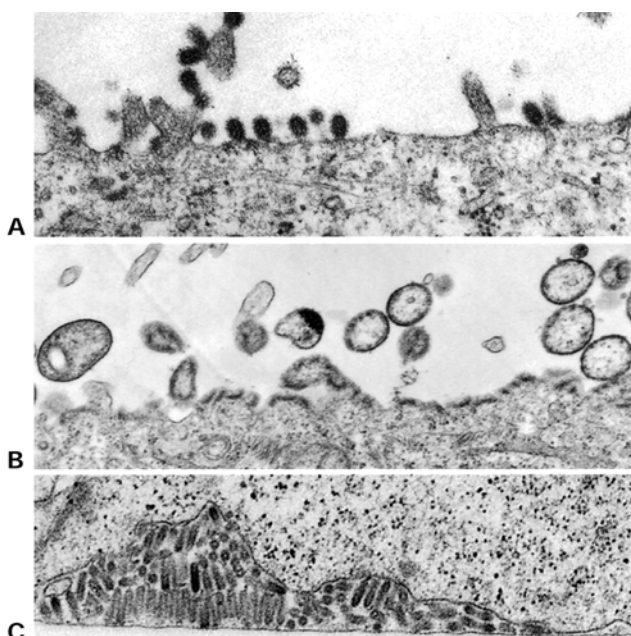
## Viral Spread

Following replication at the site of entry, virus particles can remain localized, or can spread to other tissues (Table 1). Local spread of the infection in the epithelium occurs when newly released virus infects adjacent cells. These infections are usually contained by the physical constraints of the tissue and brought under control by the intrinsic and immune defenses. An infection that spreads beyond the primary site of infection is called **disseminated**. If many organs become infected, the infection is described as **systemic**. For an infection to spread beyond the primary site, physical and immune barriers must be breached. After crossing the epithelium, virus particles reach the basement membrane (Fig. 5). The integrity of that structure may be compromised by epithelial cell destruction and inflammation. Below the basement membrane are sub-epithelial tissues, where the virus encounters tissue fluids, the lymphatic system, and phagocytes. All three play significant roles in clearing foreign particles, but also may disseminate infectious virus from the primary site of infection.



**Figure 5. View of the intestinal wall, showing a typical M cell surrounded by enterocytes.**

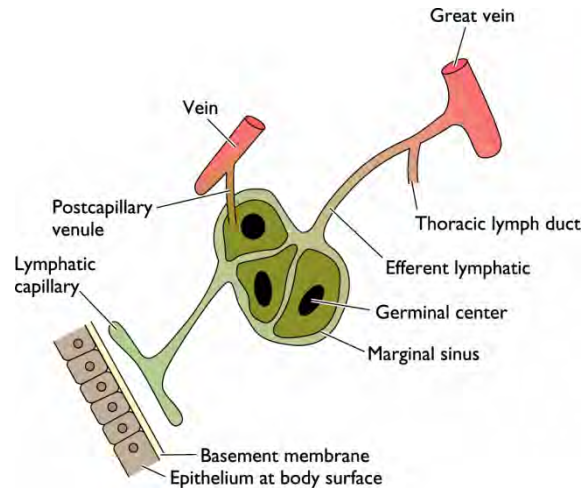
One important mechanism for avoiding local host defenses and facilitating spread within the body is the directional release of virus particles from polarized cells at the mucosal surface. Virions can be released from the apical surface, from the basolateral surface, or from both (Fig. 6). After replication, virus released from the apical surface is outside the host. Such directional release facilitates the dispersal of many newly replicated enteric viruses in the feces (e.g., poliovirus). In contrast, virus particles released from the basolateral surfaces of polarized epithelial cells have been moved away from the defenses of the luminal surface. Directional release is therefore a major determinant of the infection pattern. In general, viruses released at apical membranes establish a localized or limited infection. Release of viruses at the basal membrane provides access to the underlying tissues and may facilitate systemic spread.



**Figure 6. Polarized release of viruses from cultured cells visualized by electron microscopy.**  
A, influenza virus, apical release; B, measles virus, apical release; C, vesicular stomatitis virus, basolateral release

### *Hematogenous Spread*

Viruses that escape from local defenses to produce a disseminated infection often do so by entering the bloodstream (**hematogenous spread**). Virus particles may enter the blood directly through capillaries, by replicating in endothelial cells, or through inoculation by a vector bite. Once in the blood, viruses may access almost every tissue in the host. Hematogenous spread begins when newly replicated particles produced at the entry site are released into the extracellular fluids, which can be taken up by the local lymphatic vascular system (Fig. 7). Lymphatic capillaries are considerably more permeable than circulatory system capillaries, facilitating virus entry. As the lymphatic vessels ultimately join with the venous system, virus particles in lymph have free access to the bloodstream. In the lymphatic system, virions pass through lymph nodes, where they encounter migratory cells of the immune system. Viral pathogenesis resulting from the direct infection of immune system cells (e.g., human immunodeficiency virus, measles virus) is initiated in this fashion. Some viruses replicate in the infected lymphoid cells, and progeny are released into the blood plasma. The infected lymphoid cell may also migrate away from the local lymph node to distant parts of the circulatory system.



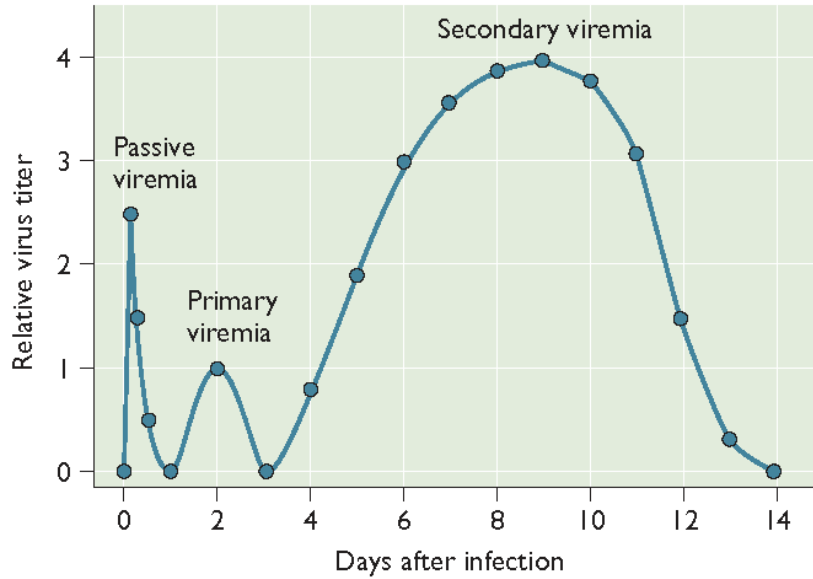
**Figure 7. The lymphatic system.**

**Table 2. Cell associated and plasma viremia**

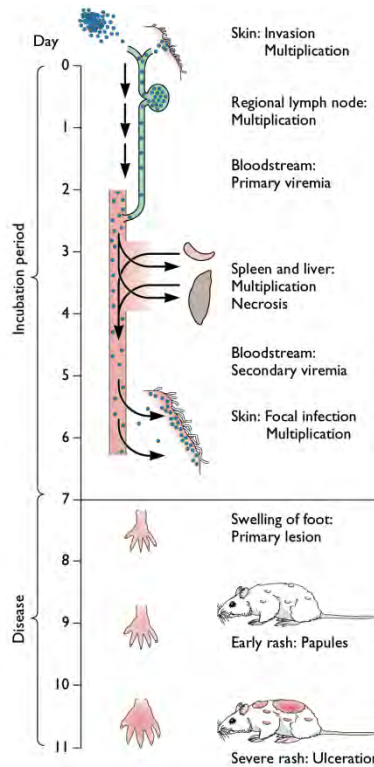
Cell type	Virus	Duration of viremia
Monocyte/macrophage	Dengue virus	Acute
	Rubella virus	Persistent
	Measles virus	Acute
	Lymphocytic choriomeningitis virus	Persistent
	Human immunodeficiency virus	Persistent
	Cytomegalovirus	Acute
B lymphocytes	Mousepox virus	Acute
	Murine leukemia virus	Persistent
	Epstein-Barr virus	Persistent
T lymphocytes	Human immunodeficiency virus	Persistent
	Human T-cell leukemia virus type 1	Persistent
	Human herpesviruses 6 and 7	Acute
Erythrocytes	Colorado tick fever virus	Acute
Neutrophils	Influenza virus	Acute
Free in plasma	Poliovirus	Acute
	Yellow fever virus	Acute
	Hepatitis B virus	Persistent

The term **viremia** describes the presence of infectious virus particles in the blood. These virions may be free in the blood or contained within infected cells such as lymphocytes (Table 2).

**Active viremia** is produced by virus replication, while **passive viremia** results when virus particles are introduced into the blood without viral replication at the site of entry (injection of a virus suspension into a vein) (Fig. 8). Progeny virions released into the blood after initial replication at the site of entry constitute **primary viremia**. The concentration of virus particles during primary viremia is usually low. However, the subsequent disseminated infections that result are often extensive, releasing considerably more virus particles. Such delayed appearance of a high concentration of infectious virus in the blood is termed **secondary viremia**. The two phases of viremia were first described in classic studies of mousepox (Fig. 9).



**Figure 8. Active and passive viremia.**



**Figure 9. Pathogenesis of mousepox.**



Viremias are of diagnostic value and can be used to monitor the course of infection, but they also present practical problems. Infections can be spread inadvertently in the population when pooled blood from thousands of individuals is used directly for therapeutic purposes (transfusions) or as a source of therapeutic proteins (e.g., gamma globulin or blood-clotting factors).

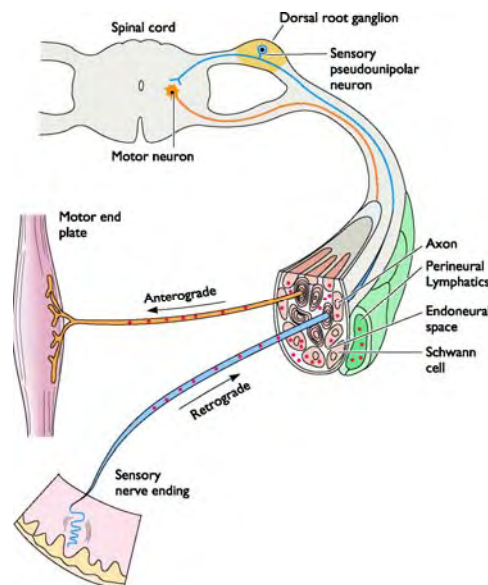
### Neural Spread

Many viruses spread from the primary site of infection by entering local nerve endings (Table 3). For certain viruses (e.g., rabies virus and alpha herpesviruses), neuronal spread is the definitive characteristic of their pathogenesis. For other viruses (e.g., poliovirus and reovirus), invasion of the nervous system is a less frequent diversion from their site of replication and destination. Some viruses (e.g., mumps virus, human immunodeficiency virus, and measles virus) may replicate in the brain, but spread is by the hematogenous route.

**Table 3. Viral spread to the central nervous system**

Pathway	Viruses
Neural	Poliovirus, yellow fever virus, mouse hepatitis virus, Venezuelan encephalitis virus, rabies virus, reovirus (type 3 only; type 1 spread by viremia), herpes simplex virus types 1 and 2, pseudorabies virus
Olfactory	Poliovirus (experimental), herpes simplex virus, coronavirus
Hematogenous	Poliovirus, coxsackievirus, arenavirus, mumps virus, measles virus, herpes simplex virus, cytomegalovirus

Because protein synthesis does not occur in the extended processes of neuronal cells, virus particles must be transported over relatively long distances to the site of viral replication. All evidence indicates that viruses are carried in the infected neuron by cellular systems, but viral proteins may facilitate the direction of spread. (Fig. 10)



**Figure 10. Pathways for the spread of viruses in nerves.**

A **neurotropic** virus can infect neural cells; infection may occur by neural or hematogenous spread initiating from a peripheral site. A **neuroinvasive** virus can enter the central nervous system (spinal cord and brain) after infection of a peripheral site. A **neurovirulent** virus can cause disease of nervous tissue, manifested by neurological symptoms and often death.

Examples:

Herpes simplex virus has low neuroinvasiveness of the central nervous system, but high neurovirulence. It always enters the peripheral nervous system but rarely enters the central nervous system. When it does, the consequences are almost always severe, if not fatal.

Mumps virus has high neuroinvasiveness but low neurovirulence. Most infections lead to invasion of the central nervous system, but neurological disease is mild.

Rabies virus has high neuroinvasiveness and high neurovirulence. It readily infects the peripheral nervous system and spreads to the central nervous system with 100% lethality unless antiviral therapy is administered shortly after infection.

## Organ Invasion

Once virions enter the blood and are dispersed from the primary site, any subsequent replication requires invasion of new cells and tissues. Three main types of blood vessel-tissue junctions provide routes of tissue invasion (Fig. 11).

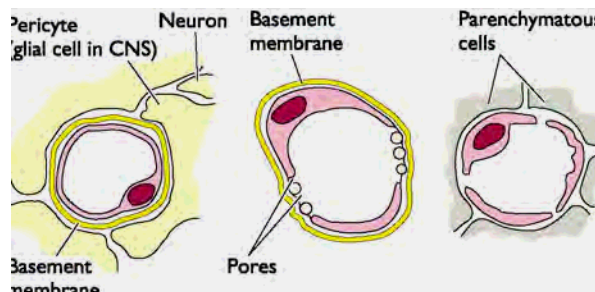


Figure 11. Three types of blood-tissue junctions: capillary, venule, and sinusoid.

## Skin

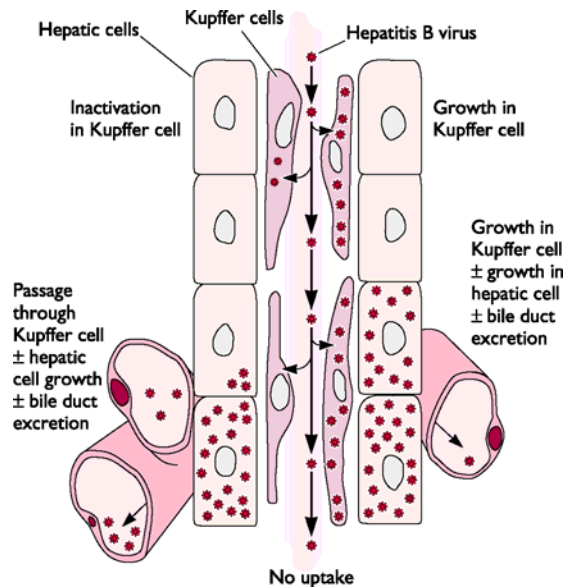
In some systemic viral infections, rashes occur when virions leave blood vessels (Table 4). Different types of skin lesions are produced. **Macules** and **papules** develop when inflammation occurs in the dermis, with the infection confined in or near the vascular bed. Vesicles and pustules occur when viruses spread from the capillaries to the superficial layers of the skin. Destruction of cells by virus replication is the primary cause of lesions.

Table 4. Viruses that cause skin rashes in humans.

Virus	Disease	Feature
Coxsackievirus A16	Hand-foot-and-mouth disease	Maculopapular rash
Echoviruses 4, 6, 9, 16; coxsackieviruses A9, 16, 23	Rash (not distinguishable)	Maculopapular rash
Measles virus	Measles	Maculopapular rash
Parvovirus	Erythema infectiosum	Maculopapular rash
Rubella virus	German measles	Maculopapular rash
Varicella-zoster virus	Chickenpox, zoster	Vesicular rash

### ***The Liver, Spleen, Bone Marrow, and Adrenal Glands***

These tissues are characterized by the presence of sinusoids lined with macrophages. Such macrophages, known as the reticuloendothelial system, function to filter the blood and remove foreign particles. They often provide a portal of entry into various tissues. For example, viruses that infect the liver usually enter from the blood. The presence of virus particles in the blood invariably leads to the infection of **Kupffer cells**, the macrophages that line the liver sinusoids (Fig. 12).

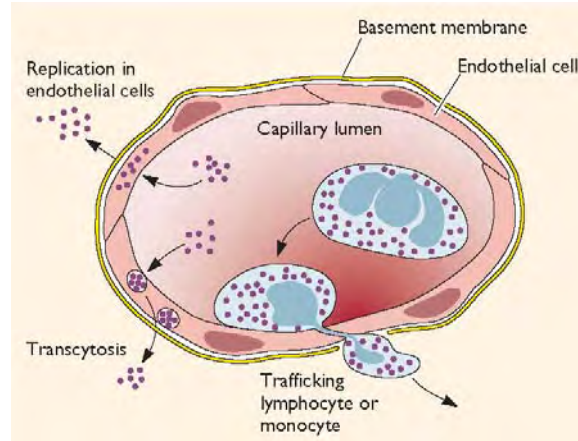


**Figure 12. Routes of viral entry into the liver.**

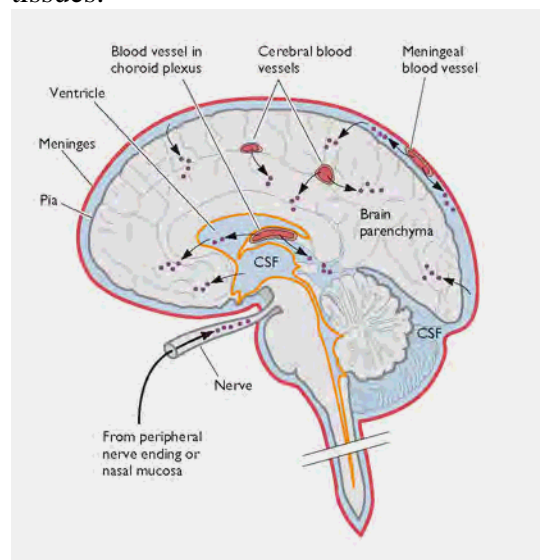
Virions may be transcytosed across Kupffer and endothelial cells without replication to reach the underlying hepatic cells. Alternatively, viruses may multiply in Kupffer and endothelial cells and then infect underlying hepatocytes. Either mechanism may induce inflammation and necrosis of liver tissue, a condition termed **hepatitis**.

### ***Central Nervous System, Connective Tissue, and Skeletal and Cardiac Muscle***

In these tissues, capillary endothelial cells are generally not fenestrated and are backed by a dense basement membrane (Fig. 12 and 13). Much study has been devoted to determining how viruses enter the brain from the blood. Routes of virus invasion into the central nervous system are summarized in Fig. 14.



**Figure 13. How viruses move from blood to tissues.** Schematic of a capillary illustrating different pathways by which viruses may leave the blood and enter underlying tissues.



**Figure 14. Summary of how viruses access the central nervous system.**

In several well-defined parts of the brain, the capillary epithelium is fenestrated and the basement membrane is sparse. These highly vascularized sites include the choroid plexus. Some viruses (e.g., mumps virus and certain togaviruses) pass through the capillary endothelium and enter the stroma of the choroid plexus, where they may cross the epithelium into the cerebrospinal fluid by either transcytosis, or replication and directed release. Once in the cerebrospinal fluid, these viruses infect the ependymal cells lining the ventricles and invade the underlying brain tissue. Other viruses may directly infect, or be transported across, the capillary endothelium (e.g., picornaviruses and togaviruses). Some viruses cross the endothelium within infected monocytes or lymphocytes (e.g., human immunodeficiency virus and measles virus,). Increased local permeability of the capillary endothelium caused, for example, by certain hormones, may also permit virus entry into the brain and spinal cord.

### ***The Renal Glomerulus, Pancreas, Ileum, and Colon***

To enter tissues that lack sinusoids (Fig. 11), viruses must first adhere to the endothelial cells lining capillaries or venules, where the blood flow is slowest and the walls are thinnest. Once blood-borne viruses have adhered to the vessel wall, they can invade readily the renal glomerulus, pancreas, ileum, or colon because the endothelial cells that make up the capillaries are fenestrated (with “windows” between cells; loosely joined together), permitting the virus or virus-infected cells to cross into the underlying tissues (e.g., poliovirus). Some viruses (e.g., herpes simplex, yellow fever, and measles viruses) cross the endothelium while being carried by infected monocytes or lymphocytes in a process called **diapedesis**.

### ***The Fetus***

In a pregnant female, viremia may lead to infection of the developing fetus (Table 5). The basement membrane is less well developed in the fetus, and infection can occur by invasion of the placental tissues and then fetal tissue. Infected circulating cells such as monocytes may enter the fetal bloodstream directly. Virus may also be transmitted to the baby during delivery or breast-feeding.

**Table 5. Congenital viral infections.**

Syndrome	Virus	Fetus
Fetal death and abortion	Smallpox virus	Human
	Parvovirus	Human
	Various alphaherpesviruses	Swine, horses, cattle
Congenital defects	Cytomegalovirus	Human
	Rubella virus	Human
Immunodeficiency	Human immunodeficiency virus type 1	Human
Inapparent (lifelong carrier)	Lymphocytic choriomeningitis virus	Mouse
	Noncytopathic bovine viral diarrhea virus	Cattle
	Murine leukemia virus	Mouse
	Avian leukosis virus	Chicken

### **Tropism**

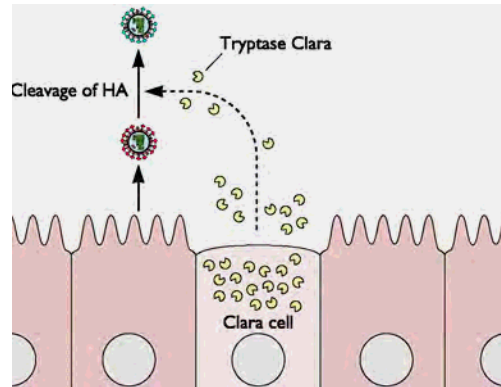
Most viruses do not infect all the cells of a host but are restricted to specific cell types of certain organs. The spectrum of tissues infected by a virus is called **tropism**. For example, an **enterotropic** virus replicates in the gut, whereas a **neurotropic** virus replicates in cells of the nervous system. Some viruses are **pantropic**, infecting and replicating in many cell types and tissues.

Tropism is governed by at least four parameters. It can be determined by the distribution of receptors for entry (**susceptibility**), or by a requirement of the virus for differentially expressed intracellular gene products to complete the infection (**permissivity**). However, even if the cell is permissive and susceptible, infection may not occur because the virus is physically prevented from interacting with the tissue (**accessibility**). Finally, an infection may not occur even when the tissue is accessible and the cells are susceptible and permissive because of the local intrinsic and innate immune defenses. In most cases, tropism is determined by a combination of two or more of these parameters.

### ***Cellular Proteases***

Many viruses require cellular proteases to cleave viral proteins to form the mature infectious virus particle. A cellular protease cleaves the influenza virus HA precursor into two subunits so that fusion of the viral envelope and cell membrane can proceed. In mammals, the replication of

influenza virus is restricted to the epithelial cells of the upper and lower respiratory tract. The tropism of this virus is influenced by the limited expression of the protease that processes HA. This serine protease, called tryptase Clara, is secreted by non-ciliated Clara cells of the bronchial and bronchiolar epithelia (Fig. 15).



**Figure 15. Cleavage of influenza virus HA0 by tryptase Clara.**

## **Viral Virulence**

Virulence refers to the capacity of a virus to cause disease in an infected host. It is a quantitative statement of the degree or extent of pathogenesis. In general, a **virulent** virus causes significant disease, whereas an **avirulent** or **attenuated** virus causes no or reduced disease, respectively.

## **Measuring Viral Virulence**

Virulence can be quantified in a number of ways. One approach is to determine the concentration of virus that causes death or disease in 50% of the infected animals. This parameter is called the 50% lethal dose (LD<sub>50</sub>), the 50% paralytic dose (PD<sub>50</sub>), or the 50% infectious dose (ID<sub>50</sub>), depending on the parameter that is measured. Other measurements of virulence include mean time to death or appearance of symptoms, and measurement of fever or weight loss. Virus-induced tissue damage can be measured directly by examining histological sections or the blood. The safety of live attenuated poliovirus vaccine is determined by assessing the extent of pathological lesions in the central nervous system in experimentally inoculated monkeys. The reduction in blood concentration of CD4<sup>+</sup> lymphocytes caused by human immunodeficiency virus type 1 infection is another example. Indirect measures of virulence include assays for liver enzymes (alanine or aspartate amino-transferases) that are released into the blood as a result of virus-induced liver damage.

## Genetic Determinants of Virulence

A major goal of animal virology is to identify viral and host genes that control virulence. To identify viral virulence genes, it is necessary to compare viruses that differ only in their degree of virulence. Before the era of modern virology, several approaches were used to attain this goal. Occasionally, natural isolates of some avirulent viruses could be identified. More recently, rapid sequencing of entire viral genomes, polymerase chain reaction (PCR) amplification of selected genomic segments, and site-directed mutagenesis have become routine procedures in the quest to identify viral virulence genes and their products.

Viral virulence genes can be placed in one of four general classes.

**1. Gene products that alter the ability of the virus to replicate.** Genes that encode proteins affecting both viral replication and virulence can be placed in one of two subclasses (Fig. 16). Viral mutants of one subclass exhibit reduced or no replication in the animal host and in many cultured cell types. Reduced virulence results from failure to produce sufficient numbers of virus particles to cause disease. Such a phenotype may be caused by mutations in any viral gene.

Mutants of the second subclass exhibit impaired virulence in animals, but no replication defects in cells in culture (except perhaps in cell types representative of the tissue in which disease develops). Such host range mutants should provide valuable insight into the basis of viral virulence, because they identify genes specifically required for disease.

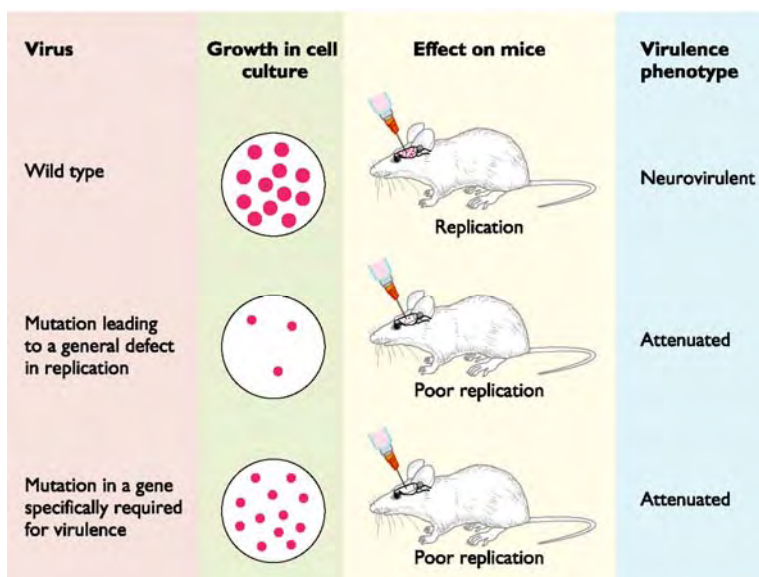


Figure 16. Different types of virulence genes.

**2. Gene products that modify the host's defense mechanisms.** The study of viral virulence genes has identified a diverse array of viral proteins that sabotage the body's intrinsic defenses and innate and adaptive systems. Some of these viral proteins are called **virokines** (secreted proteins that mimic cytokines, growth factors, or similar extra-cellular immune regulators) or **viroceptors** (homologs of host receptors). Mutations in genes encoding either class of protein affect virulence, but these genes are **not** required for growth in cell culture. Most virokines and viroceptors have been discovered in the genomes of large DNA viruses (Fig. 17).

### 3. Genes that enable the virus to spread in the host.

The mutation of some viral genes disrupts spread from peripheral sites of inoculation to the organ where disease occurs. For example, after intramuscular inoculation in mice, reovirus type 1 spreads to the central nervous system through the blood, while type 3 spreads by neural routes. Studies of viral recombinants between types 1 and 3 indicate that the gene encoding the viral outer capsid protein s1, which recognizes the cell receptor, determines the route of spread.

**4. Toxic viral proteins.** Some viral gene products cause cell injury directly, and alterations in these genes reduces viral virulence. Evidence of their intrinsic activity is usually obtained by adding purified proteins to cultured cells, or by synthesis of the proteins from plasmids or viral vectors. The most convincing example of a viral protein with intrinsic toxicity relevant to the viral disease is the NSP4 protein of rotaviruses, which cause gastroenteritis and diarrhea.

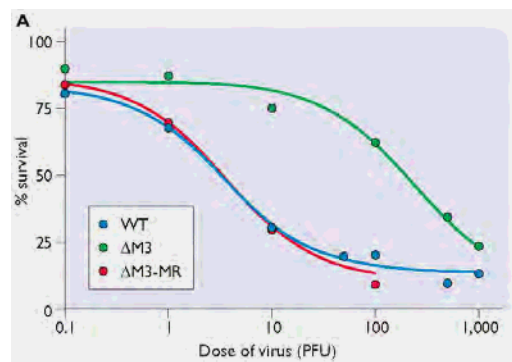
NSP4 is a non-structural glycoprotein that participates in the formation of a transient envelope as the particles bud into the endoplasmic reticulum. When this protein is produced in insect cells, it causes an increase in intracellular calcium concentration. When fed to young mice, NSP4 causes diarrhea by potentiating chloride secretion. NSP4 acts as a viral enterotoxin and triggers a signal transduction pathway in the intestinal mucosa (Figure 18).

### Injury caused by viral infections

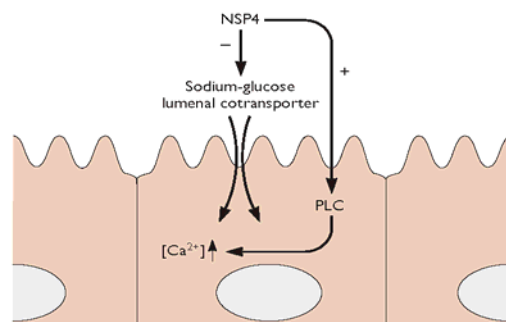
The clinical symptoms of viral disease in the host (e.g., fever, tissue damage, aches, pains, nausea) result primarily from the host response to infection. This response is initiated by cell injury caused by viral replication. Cell injury can result from the direct effects of viral replication on the cell, or from the consequences of the host's intrinsic, innate, and adaptive immune responses.

#### Direct Effects of Primary Infection

Infection of cultured cells may result in visible changes in the cells collectively called **cytopathic effect**. Direct alteration of the cell can clearly account for some of the damage observed during infections in an animal host. For example, poliovirus induces cytopathic effects in cultured neuronal cells. The relevance of this finding may be that virus-induced killing of neurons in the central nervous system could account for the paralytic symptoms characteristic of poliomyelitis. Because virus-induced cytopathic effects are clearly relevant to



**Figure 17. Role of a herpesviral chemokine in pathogenesis.** Survival of mice after intracerebral inoculation with wild type gammaherpesvirus type 68, with a mutant virus lacking the M3 gene ( $\Delta$ 3), which encodes a protein that binds CC chemokines, or with the mutant virus to which the M3 gene has been restored ( $\Delta$ 3-MR).



**Figure 18. Model for rotavirus induced diarrhea.** nsP4, produced during rotavirus replication in intestinal epithelial cells, inhibits the sodium-glucose luminal cotransporter. Because this transporter is required for water reabsorption in the intestine, its inhibition by nsP4 could be one mechanism of diarrhea induction. nsP4 also induces a phospholipase C (PLC) - dependent calcium signaling pathway. The increase of intracellular calcium could induce calcium-dependent chloride secretion.



viral pathogenesis, much effort has been devoted to understanding how infection alters cell structure and metabolism. One major process that leads to visible cell damage is apoptosis.

The consequences of viral infection may also include cessation of essential host processes such as translation, DNA and RNA synthesis, and vesicular transport. A general outcome may be the increased permeability of cell membranes. Lysosomal contents diffuse into the cytoplasm, resulting in autolytic digestion of the cell.

The host genome can be damaged directly by viral infection. For example, the retrovirus replication cycle requires insertion of a proviral DNA copy into random locations in the cell genome. Such insertion may affect the expression or integrity of a cellular gene, a process known as **insertional mutagenesis**.

### **Immunopathology: too much of a good thing.**

Most symptoms and many diseases caused by viral infection are a consequence of the immune response (Table 6). Damage caused by the immune system is called **immunopathology**, and it may be the price paid by the host to eliminate a viral infection. For non-cytolytic viruses it is likely that the immune response is the sole cause of disease.

**Table 6. Examples of virus-induced immunopathology.**

Proposed mechanism	Virus	
CD8 <sup>+</sup> T cell mediated	Coxsackievirus B	
	Lymphocytic choriomeningitis virus	
	Sin Nombre virus	
	Human immunodeficiency virus type 1	
	Hepatitis B virus	
CD4 <sup>+</sup> T cell mediated		
	Th1	Theiler's virus
		Mouse coronavirus
		Semliki Forest virus
		Measles virus
		Visna virus
		Herpes simplex virus
Th2	Respiratory syncytial virus	
Antibody mediated	Dengue virus	
	Feline infectious peritonitis virus	

### ***Immunopathological Lesions***

*Lesions caused by cytotoxic T lymphocytes (CTLs).* Myocarditis (inflammation of the heart muscle) caused by coxsackievirus B infection of mice requires the presence of CTLs. In particular, perforin is a major determinant of myocarditis. Mice lacking the perforin gene develop a mild form of heart disease yet are still able to clear the infection. The recently recognized acute and often fatal respiratory disease caused by hantaviruses is characterized by prominent infiltration of CTLs into the lung.

*Lesions caused by CD4<sup>+</sup> T-cells.* CD4<sup>+</sup> T lymphocytes elaborate far more cytokines than do CTLs and recruit and activate many nonspecific effector cells. Such inflammatory reactions are usually called delayed-type hypersensitivity responses. Most of the recruited cells are neutrophils and mononuclear cells, which are protective and cause tissue damage. Immunopathology is the result of release of proteolytic enzymes, reactive free radicals such as peroxide and nitric oxide (see below), and cytokines such as TNF- $\alpha$ .

*CD4+ Th1 T-cells.* Herpes stromal keratitis, one of the most common causes of vision impairment in developed countries of the world, is an example of an cytolytic infection in which the pathology is mainly immune-mediated. In humans, herpes simplex virus infection of the eye induces lesions on the corneal epithelium, and repeated infections result in opacity and reduced vision. Studies of a mouse model for this disease have demonstrated the importance of CD4+ Th1 T-cells. Mice lacking T-cells do not develop ocular disease following infection. Lymphocytes isolated from the corneas of mice with active disease are CD4+ T-cells that produce cytokines typical of the Th1 subset.

*CD4+ Th2 cells.* Respiratory syncytial virus disease may be mediated by CD4+ Th2 cells. This non-cytopathic virus is an important cause of lower respiratory tract disease in infants and the elderly. In immunosuppressed mice, lesions of the respiratory tract are minor, but become severe after transfer of viral antigen-specific T-cells, particularly CD4+ Th2 cells. Lesions in the respiratory tract contain many eosinophils, which may be responsible for pathology. Products of CD4+ Th2 T-cells may recruit these eosinophils.

*Immunopathological Lesions Caused by B-cells.* Virus-antibody complexes accumulate to high concentrations when extensive viral replication occurs at sites inaccessible to the immune system or continues in the presence of an inadequate immune response. Such complexes are not efficiently cleared by the reticuloendothelial system and continue to circulate in the blood. They become deposited in the smallest capillaries and cause lesions that are exacerbated when the complement system is activated. Deposition of these immune complexes in blood vessels, kidney, and brain may result in vasculitis, glomerulonephritis, and mental confusion, respectively.

Antibodies may also enhance viral infection. This mechanism probably accounts for the pathogenesis of dengue hemorrhagic fever. This disease is transmitted by mosquitoes and is endemic in the Caribbean, Central and South America, Africa, and Southeast Asia, where billions of people are at risk. The primary infection is usually asymptomatic, but may result in a self-limiting acute febrile illness with severe headache, back and limb pain, and a rash. There are four viral serotypes and antibodies to any one of these does not protect against infection by the other. After infection by another serotype of dengue virus, non-protective antibodies bind virus particles and facilitate uptake into normally non-susceptible peripheral blood monocytes carrying Fc-receptors. Consequently, the infected monocytes produce pro-inflammatory cytokines, which in turn stimulate T-cells to produce more cytokines. This vicious cycle results in high concentrations of cytokines and other chemical mediators that are believed to trigger the plasma leakage and hemorrhage characteristic of dengue hemorrhagic fever. There may be so much internal bleeding that the often-fatal dengue shock syndrome results. Dengue hemorrhagic fever occurs in approximately 1 in 14,000 primary infections. However, after infection with a dengue virus of another serotype, the incidence of hemorrhagic fever increases dramatically to 1 in 90, and the shock syndrome is seen in as many as 1 in 50.

*Injury Mediated by Free Radicals.* Nitric oxide (NO) is produced in virus-infected tissues during inflammation as part of the innate immune response. This gas has been shown to inhibit the replication of many viruses in cultured cells and in animal models. While low concentrations of NO have a protective effect, high concentrations or prolonged production have the potential to contribute to tissue damage. For example, treating infected animals with inhibitors of nitric oxide synthase prevents tissue damage. Although NO is relatively inert, it rapidly reacts with  $O_2^{\bullet}$  to form peroxynitrite ( $ONOO^{\bullet}$ ), which is much more reactive and may be responsible for cytotoxic effects on cells (Fig. 19).

