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Viral infection is not synonymous with disease, as many viral infections are subclinical (syn., asymptomatic, inapparent), whereas others result in disease of varying severity that is typically accompanied by characteristic clinical signs in the affected host (Fig. 3.1). Amongst many other potentially contributing factors, the outcome of the virus-host encounter is essentially the product of the virulence of the infecting virus on the one hand and the susceptibility of the host on the other. The term virulence is used as a quantitative or relative measure of the pathogenicity of the infecting virus—that is, a virus is said to be either pathogenic or nonpathogenic, but its virulence is stated in relative terms (“virus A is more virulent than virus B” or “virus strain A is more virulent in animal species Y than species Z”). The terms pathogenicity and virulence refer to the capacity of a virus to cause disease in its host, and are unrelated to the infectivity or transmissibility (contagiousness) of the virus.

For viruses to cause disease they must first infect their host, spread within the host, and damage target tissues. To ensure their propagation, viruses must then be transmitted to other susceptible individuals—that is, they must be shed within secretions or excretions into the environment, be taken up by another host or a vector, or be passed congenitally from mother to offspring. Viruses have developed a remarkable variety of strategies to ensure their own survival. Similarly, individual viruses cause disease through a considerable variety of distinct pathogenic mechanisms.

INTERPLAY OF VIRAL VIRULENCE AND HOST RESISTANCE, OR SUSCEPTIBILITY FACTORS IN MANIFESTATION OF VIRAL DISEASES

Viruses differ greatly in their virulence, but even in a population infected by a particular virus strain there are usually striking differences in the outcome of infection between individual animals. Similarly, there is much variation amongst viruses of the same species and the determinants of viral virulence are often multigenic, meaning that several viral genes contribute to the virulence of individual viruses. The determinants of host resistance/susceptibility are usually multifactorial, and include not only a variety of host factors but environmental ones as well.

The advent and application of molecular technologies has facilitated mapping of virulence determinants in the genome of many viruses (eg, by whole-genomic sequencing of virus strains, and manipulation of molecular clones), as well as resistance/susceptibility determinants in the genome of experimental animals. Virus strain
differences may be quantitative, involving the rate and yield of virus replication, lethal dose, infectious dose, the number of cells infected in a given organ, or they may be qualitative, involving organ or tissue tropism, extent of host-cell damage, mode and efficacy of spread in the body, and character of the disease they induce.

Assessment of Viral Virulence

There is wide variation in the virulence of viruses, ranging from those that almost always cause inapparent infections, to those that usually cause disease, to those that usually cause death. Meaningful comparison of the virulence of viruses requires that factors such as the infecting dose of the virus and the age, sex, and condition of the host animals and their immune status be equal; however, these conditions are never met in nature, where heterogeneous, outbred animal populations are the rule and the dynamics of exposure and viral infection are incredibly varied. Hence, subjective and vague terminology may be used to describe the virulence of particular viruses in domestic and wild animals. Precise measures of virulence are usually derived only from assays in inbred animals such as mice. Of course, such assays are only feasible for those viruses that grow in mice, and care must always be exercised in extrapolating data from laboratory mice to the host species of interest.

The virulence of a particular strain of virus administered in a particular dose, by a particular route, to a particular age and strain of laboratory animal may be assessed by determining its ability to cause disease, death, specific clinical signs, or lesions. The dose of the virus required to cause death in 50% of animals (lethal dose 50, LD50) has been a commonly used measure of virulence, but is now passing out of favor in the research arena for ethical reasons. For example, in the susceptible BALB/c strain of mouse, the LD50 of a virulent strain of ectromelia virus is 5 virions, as compared with 5000 for a moderately attenuated strain and about 1 million for a highly attenuated strain. Viral virulence can also be measured in experimental animals by determining the ratio of the dose of a particular strain of virus that causes infection in 50% of individuals (infectious dose 50, ID50) to the dose that kills 50% of individuals (the ID50/LD50 ratio). Thus, the ID50 of a virulent strain of ectromelia virus in BALB/c mice is 2 virions and the LD50 about 5 virions, whereas for resistant C57BL/6 mice the ID50 is the same but the LD50 is 1 million virions. The severity of an infection, therefore, depends on the interplay between the virulence of the virus and the resistance of the host. Viral virulence also can be estimated through assessment of the severity, location, and distribution of gross, histologic, and ultrastructural lesions in affected animals.

Determinants of Viral Virulence

The advent of molecular biology has facilitated determination of the genetic basis of virulence of many viruses, along with other important aspects of their replication. Specifically, the role of potential determinants of virulence identified by genetic sequence comparison of viruses of defined virulence can be confirmed unequivocally by manipulation of molecular clones of the virus in question. This “reverse genetics” strategy utilizing molecular (infectious) clones was first widely employed using complementary DNA (cDNA) copies of the entire genome of simple positive-strand RNA viruses such as alphaviruses and picornaviruses, where RNA transcribed from the full-length cDNA copies (clones) of the genomes of such viruses is itself capable of initiating the viral replication cycle following transfection into cells. The genomic RNA of negative-sense RNA viruses such as rhabdoviruses is not in and of itself infectious, but infectious virus can be recovered from cDNA clones if viral proteins supporting genome replication are also produced in cells transfected with genome-length RNA transcripts. Even the considerable logistical challenges posed by RNA viruses with segmented genomes (such as influenza viruses, bunyaviruses, arenaviruses, and reoviruses) have been overcome, and molecular clones of these viruses are now used for reverse genetic manipulation. It is also possible to specifically manipulate the genomes of even the very large DNA viruses as artificial chromosomes. Of necessity, most experimental work has been carried out in inbred laboratory animals, although molecular clones of a substantial number of pathogenic animal viruses have now been evaluated in their respective natural animal hosts. It is apparent from these reverse genetic studies that several viral genes can contribute to the virulence of individual viruses, as described under each virus family in Part II of this book.
Viruses exhibit host and tissue specificity (tropism), usually more than is appreciated clinically. Mechanistically, the organ or tissue tropism of the virus is an expression of all the steps required for successful infection, from the interaction of virus attachment molecules and their cellular receptors to virus assembly and release (see Chapter 2: Virus Replication). Organ and tissue tropisms also involve all stages in the course of infection in the whole host animal, from the site of entry, to the major target organs responsible for the clinical signs, to the site involved in virus release and shedding.

Caution should be exercised in attributing characteristics of viral epidemics solely to the virulence of the causative virus, as there typically is considerable variation in the response of individual infected animals, both within and between animal species. For example, during the epizootic of West Nile virus infection that began in North America in 1999, approximately 10% of infected horses developed neurological disease (encephalomyelitis) and, of these, some 30—35% died. Neuroinvasive disease was even less common in humans infected with this same strain of West Nile virus, whereas infected corvids (crows and their relatives) almost uniformly developed disseminated, rapidly fatal infections.

**Determinants of Host Resistance/Susceptibility**

As just described for West Nile virus, genetic differences in host resistance/susceptibility to viral infections are most obvious when different animal species are compared. Viral infections tend to be less pathogenic in their natural host species than in exotic or introduced species. For instance, myxoma virus produces a small benign fibroma in its natural host, which are wild rabbits of the Americas (*Sylvilagus* spp.), but the same virus almost invariably causes a fatal generalized infection in the European rabbit, *Oryctolagus cuniculus*. Likewise, zoonotic (transmitted from animal to human) infections caused by arenaviruses, filoviruses, paramyxoviruses, coronaviruses, and many arboviruses are severe in humans but mild or subclinical in their reservoir animal hosts.

The innate and adaptive immune responses to particular viral infections differ greatly from one individual to another (see Chapter 4: Antiviral Immunity and Virus Vaccines). Studies with inbred strains of mice have confirmed that susceptibility to specific viruses may be associated with particular major histocompatibility (MHC) antigen haplotypes, presumably because of their central role in directing the nature of the adaptive immune response generated to the infecting virus. Similarly, studies with genetically modified mice have unequivocally confirmed the critical role of innate immune responses, especially those associated with the interferon system, in conferring antiviral resistance and protection.

Expression of critical receptors on target cells is a fundamental determinant of host resistance/susceptibility to a particular virus. The more conserved or ubiquitous the receptor, the wider the host range of the virus that exploits it; for example, rabies virus, which uses sialylated gangliosides in addition to the acetylcholine receptor, has a very wide host range, but infection is restricted narrowly to a few host cell types, including myocytes, neurons, and salivary gland epithelium. Changes in viral attachment proteins can lead to the emergence of variant viruses with different tropism and disease potential. For example, porcine respiratory coronavirus arose from transmissible gastroenteritis virus, which is strictly an enteric pathogen, through a substantial deletion in the gene encoding the viral spike protein that mediates virus attachment. This change affected the tropism of the virus as well as its transmissibility.

**Physiologic Factors Affecting Host Resistance/Susceptibility**

In addition to innate and adaptive immune responses, a considerable variety of physiologic factors affect host resistance/susceptibility to individual viral diseases, including age, nutritional status, levels of certain hormones, and cell differentiation.

Viral infections tend to be most serious at both ends of life—in the very young and the very old. Rapid physiologic changes occur during the immediate postpartum period and resistance to the most severe manifestations of many intestinal and respiratory infections builds quickly in the neonate. Maturation of the immune system is responsible for much of this enhanced, age-related resistance, but physiologic changes also contribute. Malnutrition can also potentially impair immune responsiveness in adults, but it is often difficult to distinguish adverse nutritional effects from other factors found in animals living in very adverse environments.

Certain infections, particularly herpesvirus infections, can be reactivated during pregnancy, leading to abortion or perinatal infection of the progeny of infected dams. The fetus itself is uniquely susceptible to a number of different viral infections, reflecting immaturity of the immune system, immaturity of biological barriers (eg, the blood–brain barrier) and increased permissiveness of rapidly dividing cell populations, the latter being abundant in developing tissues.

Cellular differentiation and the stage of the cell cycle may affect susceptibility to infection with specific viruses. For example, paroviruses replicate only in cells that are in the late S phase of the cell cycle, so the rapidly
dividing cells of bone marrow, intestinal epithelium, and the developing fetus are vulnerable. The rapidly dividing, often migratory cell populations that occur during embryogenesis in the developing fetus are exquisitely susceptible to infection and injury by a number of viruses, notably several highly teratogenic viruses that infect the developing central nervous system (CNS).

Almost all viral infections are accompanied by fever. In classic studies of myxoma virus infection in rabbits, it was shown that increasing body temperature increased protection against disease, whereas decreasing temperature increased the severity of infection. Blocking the development of fever with drugs (eg, salicylates) increased mortality. Similar results have been obtained with ectromelia and coxsackievirus infections in mice. In contrast, fever does not accompany viral infection in certain poikilotherms (eg, fish), in which this response is probably of no or lesser selective advantage.

The immunosuppressive effects of increased concentrations of corticosteroids, whether endogenous or exogenous in origin, can reactivate latent viral infections or exacerbate active mild or subclinical viral infections, such as those caused by herpesviruses. This mechanism probably contributes to the increased incidence of severe viral infections that occurs in settings in which animals are stressed as a result of transport and/or introduction into crowded environments, such as animal shelters and feedlots. Products of host inflammatory and innate immune responses also probably contribute to the transient immunosuppression and other general signs that can accompany viral infections.

**MECHANISMS OF VIRAL INFECTION AND VIRUS DISSEMINATION**

At the level of the cell, infection by viruses (see Chapters 1 and 2: The Nature of Viruses and Virus Replication) is quite different from that caused by bacteria and other microorganisms, whereas at the level of the whole animal and animal populations there are more similarities than differences. Like other microorganisms, viruses must gain entry into their host’s body before they can exert their pathogenic effects; entry of virus into the host can occur through any of a variety of potential routes, depending on the properties of the individual virus (Table 3.1).

| Routes of Virus Entry |
|-----------------------|
| Viruses are obligate intracellular parasites that are transmitted as inert particles. To infect its host, a virus must first attach to and infect cells at one of the body surfaces, either the integument or a mucosal surface. The skin that covers the animal body externally has a relatively impermeable outer layer of keratin, and initiation of infection may require that this barrier be compromised or even bypassed via a wound such as a needle stick, insect or animal bite. Barriers to the initiation of infection on mucosal surfaces are much less formidable, specifically on the mucosal epithelial lining of the respiratory, gastrointestinal, and urogenital tracts and the nonkeratinized epithelial lining of the conjunctiva and cornea of the eyes. In animals without significant areas of keratinized epithelium (eg, fish), the skin and gills serve as an extensive mucosal surface that is the initial site of infection with many viruses. Virus replication may subsequently be limited to the body surface through which the virus entered or the virus may be disseminated to replicate in multiple tissues, with subsequent shedding from body surfaces that are either the same or different from the route of entry (Fig. 3.2). |

**Entry via the Respiratory Tract**

The mucosal surfaces of the respiratory tract are lined by epithelial cells that can potentially support the replication of viruses, so defenses are necessary to minimize the risk of infection. The respiratory tract from the nasal passages to the distal airways in the lungs is protected by the “mucociliary blanket,” which consists of a layer of mucus...
produced by goblet cells that is kept in continuous flow by the coordinated beating of cilia on the luminal surface of the epithelial cells that line the nasal mucosa and airways. Inhaled virions can be trapped in the viscous mucus layer and then carried by ciliary action from the nasal cavity and airways to the pharynx, where they are then swallowed or coughed out. The distance to which inhaled particles penetrate into the respiratory tract is inversely related to their size, so that larger particles (greater than 10 μm in diameter) are trapped on the mucociliary blanket lining the nasal cavity and airways and small particles (less than 5 μm in diameter) can be inhaled directly into the airspaces of the lungs (alveoli), where they are ingested by resident alveolar macrophages.

The respiratory system is also protected by innate and adaptive immune mechanisms that operate at all mucosal surfaces (see Chapter 4: Antiviral Immunity and Virus Vaccines), including specialized lymphoid aggregates that occur throughout the respiratory tree [eg, nasal-associated lymphoid tissue (NALT) and tonsils, and bronchus-associated lymphoid tissue (BALT)]. Despite its protective mechanisms, however, the respiratory tract is perhaps the most common portal of virus entry into the body. Environmental factors may enhance infection by compromising defense mechanisms. For example, exposure to ammonia vapor causes ciliary stasis, and serous effusions associated with inflammation can dilute the viscosity of the mucus layer, both of which can enhance a virus’ ability to attach to specific receptors on epithelial cells within the mucosa. After invasion, some viruses remain localized to the respiratory system or spread from cell to cell to invade other tissues, whereas many others become widely disseminated via lymphatics and/or the bloodstream.

**Entry via the Gastrointestinal Tract**

A substantial number of viruses (enteric viruses) are spread to susceptible hosts by ingestion of virus-contaminated food or drink. The mucosal lining of the oral cavity and esophagus (and forestomachs of ruminants) is relatively refractory to viral infection, with the notable exception of that overlying the tonsils, thus enteric viral infections typically begin within the mucosal epithelium of the stomach and/or intestines. The gastrointestinal tract is protected by several different defenses, including acidity of the stomach, the layer of mucus that tenaciously covers the mucosa of the stomach and intestines, the antimicrobial activity of digestive enzymes as well as that of bile and pancreatic secretions, and innate and adaptive immune mechanisms, especially the activity of defensins and secretory antibodies such as immunoglobulin (Ig) A, the latter produced by B lymphocytes in the gastrointestinal mucosa and mucosa-associated lymphoid tissues (MALTs). Despite these protective mechanisms, enteric infection is characteristic of certain viruses that first infect the epithelial cells lining the gastrointestinal mucosa or the specialized M cells that overlie intestinal lymphoid aggregates (Peyer’s patches).

In general, viruses that cause purely enteric infection, such as rotaviruses and enteroviruses, are acid and bile resistant. However, there are acid- and bile-labile viruses that cause important enteric infections; for example, transmissible gastroenteritis virus (a coronavirus) is protected during passage through the stomach of young pigs by the buffering action of suckled milk. Not only do some enteric viruses resist inactivation by proteolytic enzymes in the stomach and intestine, their infectivity is actually increased by such exposure. Thus cleavage of an outer capsid protein by intestinal proteases enhances the infectivity of rotaviruses. Whereas rotaviruses and coronavirus are major causes of viral diarrhea in animals, the great majority of enteric infections caused by enteroviruses, adenoviruses and many other viruses are typically subclinical. Some paroviruses, morbilliviruses, amongst others, can also cause gastrointestinal infection and
diarrhea, but only after reaching cells of the gastrointestinal tract in the course of generalized (systemic) infection after viremic spread.

**Entry via the Skin**

The skin is the largest organ of the body, and its dense outer layer of keratin provides a mechanical barrier to the entry of viruses. The low pH and presence of fatty acids in skin provide further protection, as do various other components of innate and adaptive immunity, including the presence of migratory dendritic cells (Langerhans cells) within the epidermis itself. Breaches in skin integrity such as insect or animal bites, cuts, punctures, or abrasions predispose to viral infection, which can either remain confined to the skin, such as the papillomaviruses, or disseminate widely. Deeper trauma can introduce viruses into the dermis and subcutis, where there is a rich supply of blood vessels, lymphatics, and nerves that can individually serve as routes of virus dissemination. Generalized infection of the skin, such as occurs in lumpy skin disease, sheeppox, and others, is the result not of localized cutaneous infection but of systemic viral spread via viremia.

One of the most efficient ways by which viruses are introduced through the skin is via the bite of arthropods, such as mosquitoes, ticks, *Culicoides* spp. (hematophagous midges or “gnats”), or sandflies. Insects, especially flies, may act as simple mechanical vectors (“flying needles”); for example, equine infectious anemia virus is spread among horses, rabbit hemorrhagic disease virus and myxoma virus are spread among rabbits, and fowlpox virus is spread among chickens in this way. However, most viruses that are spread by arthropods replicate in their vector, the defining feature of a “biological” vector. Viruses that are both transmitted by and replicate in arthropod vectors are called *arthoviruses*.

Infection can also be acquired through the bite of an animal, as in rabies, and introduction of a virus by skin penetration may be iatrogenic—that is, the result of veterinary or husbandry procedures. For example, equine infectious anemia virus has been transmitted via contaminated needles, twitches, ropes, and harnesses, and orf virus and papillomaviruses can be transmitted via ear tagging, tattooing, or virus-contaminated inanimate objects (*fomites*).

**Entry via Other Routes**

Several important pathogens (eg, several herpesviruses and papillomaviruses) are spread through the genital tract, and this is known as *venereal transmission*. Small tears or abrasions in the penile mucosa and the epithelial lining of the vagina may occur during sexual activity and facilitate transmission. The conjunctiva, although much less resistant to viral invasion than the skin, is constantly cleansed by the flow of secretion (tears) and mechanical wiping by the eyelids; some adenoviruses and enteroviruses, however, gain entry at this site, and a substantial number of viruses can be experimentally transmitted by this route.

**Host Specificity and Tissue Tropism**

The capacity of a virus to infect cells selectively in particular organs is referred to as *tropism* (either cell or organ tropism), which is dependent on both viral and host factors. At the cellular level, there must be an interaction between viral attachment proteins and matching cellular receptors. Although such interactions are usually studied in cultured cells, the situation is considerably more complex *in vivo*. Not only do some viruses require several cellular receptors/coreceptors (see Chapter 2: Virus Replication), some viruses utilize different receptors on different cells; for example, canine distemper virus uses CD150 (signaling lymphocyte activation molecule, SLAM) to infect cells of the lymphoid system, an important step in multisystemic viral spread, whereas it attaches to nectin 4 to target the epithelial cells that mediate viral shedding. Expression of receptors can be dynamic; for example, it has been shown experimentally that animals treated with neuraminidase have substantial protection against intranasal infection with influenza virus that lasts until the neuraminidase-sensitive receptors have regenerated. Receptors for a particular virus are usually restricted to certain cell types in certain organs, and only these cells can be infected. In large part, this accounts for both the tissue and organ tropism of a given virus and the pathogenesis of the disease caused by the virus.

The presence of critical receptors is not the only factor that determines whether the cell may become infected. Cells must support viral entry following receptor binding and the viral genome must be presented with factors required for transcription and genome replication. These requirements are not met by all cell types and thus represent a determinant of viral tropism. For example, parvoviruses may require extracellular proteases to activate their fusion protein, the fusion protein mediating viral entry following attachment. This is the case for Sendai virus, where specialized cells in the bronchioles of rats (Clara cells) secrete a protease required for productive viral infection of the lung. Similarly, papillomaviruses, retroviruses and several herpesviruses rely on the interaction between host proteins and viral genomic elements known as enhancers to support viral gene expression. Viral enhancers are gene activators that increase the efficiency of transcription of viral or cellular genes; specifically, they are short, often tandem-repeated sequences of nucleotides that may contain motifs representing DNA-binding sites for various cellular or viral site-specific DNA-binding proteins (transcription factors). Viral enhancers augment binding of DNA-dependent RNA
polymerase to promoters, thereby accelerating transcription. Because many of the transcription factors affecting individual enhancer sequences in viruses are restricted to particular cells, tissues, or host species, they can determine the tropism of viruses and can act as specific virulence factors. For example, the genomic DNA of papillomavirus contains enhancers that are active only in keratinocytes and, indeed, only in the subset of these cells in which papillomavirus replication occurs.

**Mechanisms of Viral Spread and Infection of Target Organs**

The ability to restrict viral infection to the body surface that is the point of entry, as contrasted to multisystemic dissemination of virus infection, has profound implications on virus shedding and thus transmission of infection within a population of susceptible animals (Fig. 3.2). From the virus’ standpoint, the challenge to local spread is the ability to infect a sufficient number of epithelial cells to support a level of shedding that assures transmission. The benefits of local spread are more limited opportunities for the immune system to disrupt the course of infection. In contrast, multisystemic spread may introduce virus to many body surfaces that can participate in shedding, and the surface area supporting replication may be much greater than can be achieved via local spread. The challenges to the virus during multisystemic spread include the numerous opportunities for the immune system to disrupt the infection cycle, the potential need to infect multiple cell types, and the need to balance cytopathic effects with the requirement for viable cells to support step-wise spread throughout the body.

In pioneering experiments in 1949, Frank Fenner used ectromelia virus (the agent of mousepox) as a model system that first revealed the sequence of events leading to systemic infection and disease. Groups of mice were inoculated in the footpad of a hind limb, and at daily intervals their organs were titrated to determine the amount of virus present. Fenner showed that, during the incubation period, infection spread through the mouse body in a step-wise fashion. The virus first replicated locally in tissues of the footpad and then in the draining lymph nodes. Virus produced in these sites then gained entry into the bloodstream, causing a primary viremia, which brought the virus to its initial target organs (organ tropism), especially the spleen, lymph nodes, and the liver. Virus produced in the target organs—ie, the spleen and liver—caused a secondary viremia that disseminated virus to the skin and mucosal surfaces. Infection in the skin caused a macular and papular rash from which large amounts of virus were shed, leading to contact exposure of other mice. Infection ultimately resulted in tissue necrosis, this being the cause of death, but not until spread within the host and shedding from the host was achieved. This pattern has subsequently been demonstrated for many viruses of veterinary medical relevance, and can be illustrated by canine distemper virus infection of a young immunologically naïve dog. Peak virus shedding occurs at the point of epithelial infection and peak viral burden in the host. Clinical signs, reflecting cumulative effects of virus replication in multiple organ systems, are not manifest until after significant virus shedding has begun. The onset of immune-mediated viral clearance correlates with the appearance of clinical signs of infection. Courtesy of M. Oglesbee and S. Niewiesk, The Ohio State University.

**FIGURE 3.3** Relationship between initiation of infection, spread, total viral load (burden), and clinical signs in a multisystemic infection, illustrated here by canine distemper virus infection of a young immunologically naïve dog. Peak virus shedding occurs at the point of epithelial infection and peak viral burden in the host. Clinical signs, reflecting cumulative effects of virus replication in multiple organ systems, are not manifest until after significant virus shedding has begun. The onset of immune-mediated viral clearance correlates with the appearance of clinical signs of infection. Courtesy of M. Oglesbee and S. Niewiesk, The Ohio State University.
with fever signaling the onset of adaptive immune responses that drive viral clearance. Death, if it occurs, reflects the combination of immune suppression and compromised mucosal barriers that facilitate secondary microbial infections (e.g., bacterial bronchopneumonia). Death may also reflect viral infection of brain, a by-product of the secondary viremia. However, these events occur only after the infection cycle is complete and shedding has occurred.

Local Spread on Epithelial Surfaces
Viruses first replicate in epithelial cells at the site of entry and produce a localized infection, often with associated virus shedding directly into the environment from these sites. The spread of infection along epithelial surfaces occurs by the sequential infection of neighboring cells, which, depending on the individual virus, may or may not precede spread into the adjacent subepithelial tissues and beyond.

In the skin, papillomaviruses and poxviruses such as orf virus remain confined to the epidermis, where they induce localized proliferative lesions, whereas other poxviruses such as lumpy skin disease virus spread widely after cutaneous infection to involve other organ systems. Viruses that enter the body via the respiratory or intestinal tracts can quickly cause extensive infection of the mucosal epithelium, thus diseases associated with these infections progress rapidly after a short incubation period. In mammals, there is little or no productive invasion of subepithelial tissues of the respiratory tract after most influenza and parainfluenza virus infections, or in the intestinal tract following most rotavirus and coronavirus infections. Although these viruses apparently enter lymphatics and thus have the potential to spread, they usually do not do so, because appropriate viral receptors or other permissive cellular factors such as cleavage-activating proteases or transcription enhancers are restricted to epithelial cells, or because of other physiological constraints.

Restriction of viral infection to an epithelial surface should never be equated with lack of virulence or disease severity. Although localized, injury to the intestinal mucosa caused by rotaviruses and coronaviruses can result in severe and, especially in neonates, even fatal diarrhea. Similarly, influenza virus infection can cause extensive injury in the lungs, leading to acute respiratory distress syndrome and possibly death.

Subepithelial Invasion and Lymphatic Spread
A variety of factors probably contribute to the ability of some viruses to breach the epithelial barrier and to invade the subepithelial tissues, including (1) targeted migration of virus within phagocytic leukocytes, specifically dendritic cells and macrophages, and (2) directional shedding of viruses from the infected epithelium (see Chapter 2: Virus Replication). Dendritic cells are abundant in the skin and at all mucosal surfaces, where they constitute a critical first line of immune defense, both innate and adaptive (see Chapter 4: Antiviral Immunity and Virus Vaccines). Migratory dendritic cells (such as Langerhans cells in the skin) “traffic” from epithelial surfaces to mucosa-associated lymphoid tissue (MALT), which would include lymphoid organs such as tonsils and Peyer’s patches, and the adjacent (draining) regional lymph node. Infection of these migratory dendritic cells may be responsible for the initial spread of alphaviruses, bluetongue, African horse sickness and other orbiviruses, and feline and simian human immunodeficiency viruses, amongst many others. Directional release of virus into the lumen of the respiratory or intestinal tracts facilitates local spread to the surface of contiguous epithelial cells and immediate shedding into the environment, whereas shedding from the basolateral cell surface of epithelial cells potentially facilitates invasion of subepithelial tissues and subsequent virus dissemination via lymphatics, blood vessels, or nerves.

Many viruses that are widely disseminated in the body following infection at epithelial surfaces are first carried to the adjacent (regional) lymph nodes through the afferent lymphatic drainage (Fig. 3.4). Within the draining lymph node, virions may be inactivated and processed by macrophages and dendritic cells so that their component antigens are presented to lymphocytes to stimulate adaptive immune responses (see Chapter 4: Antiviral Immunity and Virus Vaccines). Some viruses, however, replicate efficiently in macrophages (e.g., many retroviruses, orbiviruses, filoviruses, canine distemper virus and other morbilliviruses, arteriviruses such as porcine reproductive and respiratory syndrome virus, and some herpesviruses), and/or in dendritic cells and lymphocytes. From the regional lymph node, virus can spread to the bloodstream in efferent lymph, and then quickly be disseminated throughout the body, either within cells or as cell-free virions. Blood-filtering organs, including the lung, liver, and spleen, are often target organs of viruses that cause disseminated infections.

Normally, there is a local inflammatory response at the site of viral invasion, the severity of which reflects the extent of tissue damage. Inflammation leads to characteristic alterations in the flow and permeability of local blood vessels, as well as leukocyte trafficking and activity. Some viruses take advantage of these events to infect cells that participate in this inflammatory response, which in turn can facilitate spread of these viruses either locally or systemically. Local inflammation may be especially important to the pathogenesis of arthropod-transmitted viruses because of the marked reaction at the site of virus inoculation induced by the bite of the arthropod vector.
Spread via the Bloodstream: Viremia

The blood is the most effective vehicle for rapid spread of virus through the body. Initial entry of virus into the blood after infection is designated primary viremia, which, although usually inapparent clinically (subclinical), leads to the seeding of distant organs. Virus replication in major target organs leads to the sustained production of much higher concentrations of virus, producing a secondary viremia (Fig. 3.5) and infection in yet other parts of the body that ultimately results in the clinical manifestations of the associated disease.

In the blood, virions may circulate free in the plasma or may be contained in, or adsorbed to, leukocytes, platelets, or erythrocytes (red blood cells). Parvoviruses, enteroviruses, togaviruses, and flaviviruses typically circulate free in the plasma. Viruses carried in leukocytes, generally lymphocytes or monocytes, are often not cleared as readily or in the same way as viruses that circulate in the plasma. Specifically, cell-associated viruses may be protected from antibodies and other plasma components, and they can be carried as “passengers” when leukocytes that harbor the virus emigrate into tissues. Individual viruses exhibit tropism to different leukocyte populations; thus monocyte-associated viremia is characteristic of canine distemper, whereas lymphocyte-associated viremia is a feature of Marek’s disease and bovine leukemia. Erythrocyte-associated viremia is characteristic of infections caused by African swine fever virus and bluetongue virus. The association of bluetongue virus with erythrocytes facilitates both prolonged viremia by delaying immune clearance, and infection of the hematophagous (blood-feeding) Culicoides midges that serve as biological vectors of the virus. A substantial number of viruses, including equine infectious anemia virus, bovine viral diarrhea virus, and bluetongue virus, associate with platelets during viremia—an interaction that might facilitate infection of endothelial cells. Neutrophils, like platelets, have a very short lifespan; neutrophils also possess powerful antimicrobial mechanisms and they are rarely infected, although they may contain phagocytosed virions.

Virions circulating in the blood are removed continuously by macrophages, thus viremia can typically be maintained only if there is a continuing introduction of virus into the blood from infected tissues or if clearance by tissue macrophages is impaired. Although circulating leukocytes can themselves constitute a site for virus replication, viremia is usually maintained by infection of the parenchymal cells of target organs such as the liver, spleen, lymph nodes, and bone marrow. In some infections, such as African horse sickness virus and equine arteritis virus infections of horses, viremia is largely maintained by the infection of endothelial cells and/or macrophages and dendritic cells. Striated and smooth muscle are an uncommon site for viral replication, not representing a target organ essential to completion of the viral infection cycle within the host, but nonetheless significant from a clinical standpoint due to the clinical signs associated with inflammation of the muscle (eg, the myositis that may accompany influenza virus infections).

There is a general correlation between the magnitude of viremia generated by blood-borne viruses and their capacity to invade target tissues. Certain neurotropic viruses are virulent after intracerebral inoculation, but avirulent when given peripherally, because they do not attain viremia titers sufficient to facilitate invasion of the nervous system. The capacity to produce viremia and the capacity to invade tissues from the bloodstream are, however, two different properties of a virus. For example, some strains of Semliki Forest virus (and certain other alphaviruses) have lost the capacity to invade the CNS while retaining the capacity to generate a viremia equivalent in duration and magnitude to that produced by neuroinvasive strains.

Viruses that circulate in blood, especially those that circulate free in plasma, encounter, amongst many others, two cell types that exert especially important roles in
determining the subsequent pathogenesis of infection: macrophages and vascular endothelial cells.

**Virus Interactions with Monocytes and Macrophages**

Macrophages are bone marrow-derived mononuclear phagocytic cells that are present in all compartments of the body. Their precursors are monocytes in the blood, the largest of the leukocytes. Monocytes migrate into tissues to become part of the normal resident macrophage population found in submucosal connective tissue, spleen and bone marrow, alveoli of the lung, sinusoids of lymph nodes and liver, and parenchyma of the brain (ie, brain microglia). Monocytes also migrate into areas of inflammation to supplement the macrophage population. Macrophages are generally considered to play host protective roles in microbial infection (Fig. 3.6). They may phagocytize and thus inactivate viruses and, together with dendritic cells, have a critical role in antigen processing and presentation to other immune cells that is central to the initiation of adaptive immune responses (see Chapter 4: Antiviral Immunity and Virus Vaccines). They also initiate innate immune responses because of their

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**FIGURE 3.5** The role of viremia in the spread of viruses through the body, indicating sites of replication and important routes of shedding of various viruses. Subepithelial invasion and spread of infection is associated with a primary round of replication that leads to primary viremia. That viremia infects target organs that further amplify viral burden, resulting in a high-level secondary viremia. Secondary viremia may result in the infection of target organs that are conducive to viral shedding, transmission of infection via arthropod vectors, or infection of organs that are a dead end for transmission (eg, brain). *Courtesy of M. Oglesbee and S. Niewiesk, The Ohio State University.*
Types of interactions between viruses and monocytes and macrophages. Virus may exploit these cells to facilitate spread or to generate viral progeny following infection. Alternatively, macrophages may restrict virus replication and take on a host defense role which includes initiation of innate immune and pro-inflammatory responses. Innate immune responses include production of type 1 interferon (IFN) and presentation of viral antigen, both of which facilitate subsequent adaptive immunity to the virus. Pro-inflammatory responses include production of cytokines such as tumor necrosis factor (TNF). While these pro-inflammatory responses can mediate host protective responses, excesses can paradoxically contribute to manifestations of disease. 

Courtesy of M. Oglesbee and S. Niewiesk, The Ohio State University.

ability to detect the presence of pathogen-associated molecular patterns (“microbial signatures”) through specific receptors—eg, Toll-like receptors. Toll-like receptor signaling is an important basis for the production of type I interferons that restrict viral virulence.

In contrast to these protective roles, macrophages may contribute to the spread of virus infection and/or tissue damage. Some viruses exhibit a specific tropism for macrophages, where they replicate to high levels. Venezuelan equine encephalitis virus is one such virus, where replication of the virus in macrophages determines the level of viremia which in turn facilitates invasion of the CNS. Viral replication in macrophages may also be envisioned to reduce the contributions of these cells to innate and adaptive antiviral immune responses, thus indirectly contributing to viral burden and spread. Productive infection of macrophages may facilitate local viral spread to neighboring parenchymal cells, as has been suggested for infectious canine hepatitis virus infection of dogs where viral antigen is detected in both hepatocytes and sinusoidal macrophages (Kupffer cells) of the liver. Virus infection of macrophages may enhance inflammatory responses that contribute to tissue injury. For example, hemorrhagic viral fevers caused by Ebola and bluetongue viruses are characterized by induction of inflammatory and vasoactive mediators such as tissue necrosis factor (TNF) by macrophages and dendritic cells, and these cytokines contribute to the pathogenesis of disease. It should be emphasized that virus infection of macrophages may reflect interaction of viral attachment proteins with specific host cell receptors, or simply an indirect consequence of the phagocytic mechanisms employed by these cells. Although macrophages are inherently efficient phagocytes, this capacity is even further enhanced after their activation by certain microbial products and cytokines such as interferon-γ. Macrophages also have Fc receptors and C3 receptors that further augment their ability to ingest opsonized virions, specifically those virions that are coated with antibody or complement molecules. For viruses that are capable of replicating in macrophages, opsonization of virions by antibody can actually facilitate antibody-mediated enhancement of infection, which may be a major pathogenetic factor in human dengue and several retrovirus infections. Virus infection of monocytes should be considered in this discussion, having potential to profoundly influence viral spread by exploiting the tendency of these cells to migrate into tissues as part of an inflammatory response or simply to replenish the normal resident macrophage population. Monocyte infection is a form of cell-associated viremia that is important to the pathogenesis of lentivirus infections and a proposed mechanism for neuroinvasion by paramyxoviruses.

In many instance, the contribution of virus interaction with macrophages is more difficult to define in terms of its protective versus detrimental role to the host. Macrophages are heterogeneous in their functional activity, which can vary markedly depending on their location and state of activation; even in a given tissue or site there are subpopulations of macrophages that differ in phagocytic activity and in susceptibility to viral infection. Differences in virus—macrophage interactions may account for differences in the virulence of closely related viruses, individual strains of the same virus, and differences in host resistance.

Virus Interactions with Vascular Endothelial Cells

The vascular endothelium with its basement membrane constitutes the blood—tissue interface and may represent a barrier for particles such as virions in locations where endothelial cells are nonfenestrated and joined together by tight junctions. The degree of barrier function varies between tissue compartments, being greatest in the brain and eye. Parenchymal invasion by circulating virions depends on crossing such barriers, often in capillaries and venules, where blood flow is slowest and the vascular wall is thinnest. Virions may move passively between or through endothelial cells and the basement membrane of small vessels, be carried within infected leukocytes (so-called “Trojan horse” mechanism), or infect endothelial
cells and “grow” their way through this barrier, with infection of the luminal aspect of the cell and release from the basal aspect. This subject has been studied most intensively in relation to viral invasion of the CNS, but it also applies to invasion of many tissues during generalized infections.

Endothelial infection may be clinically inapparent, reflecting a noncytopathic infection that facilitates viral spread. Alternatively, infection of endothelial cells may be characterized by vascular injury that results in widespread hemorrhage and/or edema, contributing to the pathogenesis of the so-called hemorrhagic viral fevers. Virus-induced endothelial injury leads to vascular thrombosis and, if widespread, disseminated intravascular coagulation (consumptive coagulopathy). However, it is likely that inflammatory and vasoactive mediators produced by virus-infected macrophages and dendritic cells, such as tissue necrosis factor, also contribute to the pathogenesis of vascular injury in hemorrhagic viral fever (Fig. 3.6).

**Spread via Nerves**

Although infection of the CNS can occur after hematogenous spread, invasion via the peripheral nerves is also an important route of infection—eg, in rabies, Borna disease, and several alphaherpesvirus infections (eg, B virus encephalitis, pseudorabies, and bovine herpesvirus 5 encephalitis). Herpesviruses can travel to the CNS in axon cytoplasm and, while doing so, also sequentially infect Schwann cells of the nerve sheath. Rabies virus and Borna disease virus also travel to the CNS in axon cytoplasm, but usually do not infect the nerve sheath. Sensory, motor, and autonomic nerves may be involved in the neural spread of these viruses. As these viruses move centripetally, they must cross cell–cell junctions. Rabies virus and pseudorabies virus can efficiently traverse synaptic junctions (Fig. 3.7).

In addition to passing centripetally from the body surface to the sensory ganglia and from there to the brain, herpesviruses can move through axons centrifugally from ganglia to the skin or mucous membranes. This is the same phenomenon that occurs after reactivation of latent herpesvirus infections and the subsequent production of recrudescent epithelial lesions. Centrifugal spread through axons is also the mechanism by which rabies virus reaches salivary glands from the brainstem, with salivary gland infection being important to viral shedding.

Viruses can also use olfactory nerve endings in the nares as sites of entry, including rhabdoviruses (eg, rabies virus and vesicular stomatitis virus), herpesviruses, and paramyxoviruses. They gain entry in the special sensory endings of the olfactory neuroepithelial cells where they cause local infection and progeny virus (or subviral entities containing the viral genome) then travel in axoplasm of olfactory nerves directly to the olfactory bulb of the brain.

**Mechanisms of Virus Shedding**

Shedding of infectious virions is crucial to the maintenance of infection in populations (see Chapter 6: Epidemiology and Control of Viral Diseases). For viruses that replicate only at epithelial surfaces, exit of infectious virions usually occurs from the same organ system involved in virus entry (eg, the respiratory or gastrointestinal system; Fig. 3.2). In generalized viral infections, shedding can occur from a variety of sites (Fig. 3.5), and some viruses are shed from several sites. The amount of virus shed in an excretion or secretion is important in relation to transmission. Very low concentrations may be
irrelevant unless very large volumes of infected material are involved; however, some viruses occur in such high concentrations that a minute quantity of virus-laden secretion or excretion can readily lead to transmission to the next animal host. Enteric viruses are in general more resistant to inactivation by environmental conditions than respiratory viruses; especially when suspended in water and protected from light, such viruses can persist in the environment for some time.

Viruses such as influenza and the pneumoviruses that typically cause localized infection and injury of the respiratory tract are shed in mucus and are expelled from the respiratory tract during coughing or sneezing. Viruses are also shed from the respiratory tract in several systemic infections. Enteric viruses such as rotaviruses are shed in the feces, and the more voluminous the fluid output the greater is the environmental contamination they cause. A few viruses are shed into the oral cavity from infected salivary glands (eg, rabies virus and cytomegaloviruses) or from the lungs or nasal mucosa during infection of the respiratory system. Salivary spread depends on activities such as licking, nuzzling, grooming, or biting. Virus shedding in saliva may continue during convalescence or recurrently thereafter, especially with herpesviruses.

The skin is an important source of virus in diseases in which transmission is by direct contact or via small abrasions: papillomaviruses and some poxviruses and herpesviruses employ this mode of transmission. Although skin lesions are produced in several generalized diseases, the skin is not generally a source of significant viral shedding. Exceptions include vesicular diseases such as foot-and-mouth disease, vesicular stomatitis, and swine vesicular disease, where the causative viruses are produced in great quantities in vesicles within the mucosa and skin of affected animals; virus is shed from these lesions after the vesicles rupture. Localization of virus in the feather follicles is important in the shedding of Marek’s disease virus by infected chickens.

Urine, like feces, tends to contaminate food sources and the environment. A number of viruses (eg, infectious canine hepatitis virus, foot-and-mouth disease viruses, and the arenaviruses) replicate in tubular epithelial cells in the kidney and are shed in urine. Canine distemper virus replicates in transitional epithelium of the renal pelvis, ureters and urinary bladder, also contributing to urinary viral shedding or “viruria.” Viruria is prolonged and common in equine rhinitis A virus infection and lifelong in arenavirus infections of reservoir rodent species; it constitutes the principal mode of contamination of the environment by these viruses.

Several viruses that cause important diseases of animals are shed in the semen and are transmitted during coitus; for example, equine arteritis virus can be shed for months or years in the semen of apparently healthy carrier stallions, long after virus has been cleared from other tissues. Similarly, viruses that replicate in the mammary gland are excreted in milk, which may serve as a route of transmission—eg, caprine arthritis—encephalitis virus, mouse mammary tumor virus, and some of the tick-borne flaviviruses. In salmonid fish, the fluid surrounding eggs oviposited during spawning may contain high concentrations of viruses such as infectious hemopoietic necrosis virus, which is an important mode of virus transmission in both hatchery and wild fish populations.

Although not “shedding” in the usual sense of the word, blood and tissues from slaughtered animals must be considered important sources of viral contagion. Virus-laden blood is also the basis for transmission when it contaminates needles and other equipment used by veterinarians and others treating or handling sick animals. Similarly, the use of virus-contaminated fetal bovine serum can result in similar contamination of biological products.

**Virus Infection Without Shedding**

Many sites of virus replication might be considered “dead ends” from the perspective of natural spread. Infection of the brain may not result in shedding in the case of paramyxoviruses, although it is significant from the standpoint of clinical disease. Transmission may occur in instances where infected nervous tissues and muscle are ingested by carnivores and omnivores. Similarly, classical swine fever (hog cholera) and African swine fever have been translocated to different regions and countries through feeding garbage containing contaminated pork scraps. The prion diseases are an analogous example, where the unprecedented epizootic of bovine spongiform encephalopathy (mad cow disease) in the United Kingdom was spread widely amongst cattle by the feeding of contaminated meat and bone meal containing bovine offal that included nervous tissue.

Some viruses, notably retroviruses and bovine virus diarrhea virus are also transmitted directly in the germplasm or by infection of the avian egg or developing mammalian embryo. Despite the lack of horizontal transmission, these vertically transmitted viruses accomplish the same ends as those shed into the environment—that is, transmission to new hosts and perpetuation in nature.

**MECHANISMS OF VIRAL INJURY AND DISEASE**

The most common adaptation of a virus to a host involves infection, spread, and shedding with minimal if any adverse effects on the host. Medically relevant virus infections are distinct in that infection causes tissue injury and thus disease (Fig. 3.8). Tissue injury may facilitate
virus propagation within or transmission between hosts, and at minimum should not interfere with these processes if the virus is to be maintained within a specific population of animals. Virus-induced cytopathic effects may induce inflammatory and physiological responses such as coughing and sneezing that facilitate shedding and transmission. Induction of diarrhea is another means of facilitating transmission by enhancing environmental contamination with progeny virus. Virus-induced immune suppression may confound host attempts at clearance and thus benefit viral spread, while also predisposing the infected host to secondary microbial infections. Tissue injury may reflect host defense mechanisms that include apoptosis or immune responses that target virus-infected cells. In other instances, damage to the host may be a consequence of virus replication in which there is no known advantage to either the virus or host, or reflects a byproduct of infection with no significant impact on transmission. The latter includes many instances where viruses infect the CNS, resulting in congenital malformations in fetuses or neonates, or clinically significant inflammatory disease in older animals. Host species is a significant variable when considering the potential of a virus to cause disease, where a given virus may cause clinically inapparent infection in a reservoir species and clinical disease in a species to which the virus is less adapted. Mechanisms of virus-induced tissue injury may be considered “direct” when they are a direct consequence of virus replication within a cell or tissue, and “indirect” when the injury is mediated by a host immune or inflammatory response.

**Types of Virus—Cell Interactions**

Virus-induced tissue injury reflects viral cell and tissue tropism, and the mode of replication within the infected cells. As described in the preceding section, cellular tropism of viruses is determined by the presence of appropriate cellular receptors and an environment that is conducive to virus gene expression and replication. The latter may include the expression of cell-type-specific proteases, transcription factors, and other factors required for viral replication. Cells are said to be permissive to infection if they provide such an environment. Viruses typically encode genes that modulate host-cell functions for their own benefit and, of course, the host has elaborate innate defenses to restrict viral functions (see Chapter 4: Antiviral Immunity and Virus Vaccines). Permissiveness may thus also reflect the ability of a virus to inhibit innate antiviral defense mechanisms. Viral and cellular factors that influence the outcome of infection are often in delicate balance and easily shifted one way or the other. The dynamic nature of the virus—cell relationship is defined in terms that describe the degree of damage to the infected cell and the production of viral progeny.

**Cytopathic infections** are characterized by loss of cell functions that are essential to survival. Cell degeneration and necrosis or virus-induced apoptosis are final outcomes of cytopathic infections. These infections are alternatively described as cytocidal (meaning “cell death”) or cytolytic (meaning “cell lysis” or “rupture”). Cell lysis is required for release of nonenveloped viral progeny, whereas progeny of enveloped viruses can be released by budding from viable cells. Cell maintenance functions are preserved in noncytopathic infections. Noncytopathic infections can be clinically significant when they disrupt cell specialized functions. For example, noncytopathic infections of neurons may cause loss of impulse conduction, and noncytopathic infection of oligodendrocytes may result in loss of myelin formation, both of which contribute to clinical neurological disease despite survival of the infected cells. A noncytopathic virus—cell relationship may give rise to a persistent infection due to survival of the cell, the inability of immune mechanisms to eliminate the virus, and a low level of virus replication that assures persistence of the virus’ genetic information. Persistence may be associated with production of viral progeny (productive infections) or the absence of viral progeny (nonproductive infections), whereas cytopathic infections are generally productive. A persistent productive infection may result in viral carriers capable of
lifelong shedding, and may continually seed infections within the host and stimulate immune and inflammatory responses that contribute to chronic disease.

**Latent infection** may be viewed as a type of persistent infection in which the viral genome is not transcribed and so there is no production of viral proteins or progeny. The viral genome is maintained indefinitely in the cell, either by the integration of the viral nucleic acid into the host-cell DNA or by carriage of the viral nucleic acid in the form of an episome, and the infected cell survives and may divide repeatedly. As such, latent infections are restricted to infection by DNA viruses or RNA viruses capable of generating DNA copies of their genome. Clinical significance of these infections is that virus gene expression can be periodically reactivated, giving rise to the production of viral protein and infectious viral progeny. This is the case of neurons latently infected with herpesviruses, where reactivation results in progeny production that in turn is amplified by productive cytopathic infections of other tissues. Persistent or latent infections with oncogenic viruses may also lead to cell transformation, as described later in this chapter. The various types of interaction that can occur between virus and cell are summarized in Table 3.2 and in Fig. 3.8.

**Cytopathic Changes in Virus-Infected Cells**

Cytopathic viral infections ultimately kill the cells in which they replicate, by preventing synthesis of host macromolecules (as described below), by producing degradative enzymes or toxic products, or by inducing apoptosis. In a productive infection of tissue culture cells, the first round of virus replication yields progeny virions that spread through the medium to infect both adjacent and distant cells; all cells in the culture may eventually become infected. Cells exhibit biochemical and structural changes that are collectively referred to as a *cytopathic effect*. Some cytopathic effects have a light microscopic appearance that is characteristic of the particular virus involved, and is therefore an important preliminary clue in the identification of clinical isolates in the diagnostic laboratory (see Chapters 2 and 5: Virus Replication and Laboratory Diagnosis of Viral Infections) (Fig. 3.9). Other changes reflect disruption of cellular processes that are less specific to the infecting virus. Apoptotic cells have a characteristic light microscopic appearance, although this host defense mechanism can be elicited by members of numerous virus families. Similarly, virus-induced metabolic and toxic insults to the cell may result in morphological changes indicative of cell degeneration and necrosis, the cumulative effect of numerous insults that may be triggered by a number of different viruses. Cytopathic effects should be viewed in context of their relationship to viral replication; to what degree is the change unique to a particular group of viruses, and does the change influence the virus’ ability to produce progeny?

**Inclusion bodies** are sites of viral transcription and genome replication in the cell that are readily apparent in cells by light microscopy. DNA viruses that replicate in the nucleus utilize cell machinery to varying degrees in support of transcription and genome replication. Host cell DNA may be displaced from the nuclear matrix by the viral genome, resulting in chromatin margination along the nuclear membrane as aggregates of viral nucleic acid and protein accumulate. The result is an *intranuclear inclusion*—an aggregate of uniform staining that is distinct from nuclear structures observed in uninfected cells. Stains used routinely in diagnostic settings yield red

| Table 3.2 Types of Virus–Cell Interaction |
|------------------------------------------|
| **Type of Infection** | **Effects on Cell** | **Production of Infectious Virions** | **Examples** |
|----------------------|---------------------|---------------------------------|--------------|
| Cytocidal            | Morphologic changes in cells (cytopathic effects); inhibition of protein, RNA, and DNA synthesis; cell death | Yes | Alphaherpesviruses, enteroviruses, reoviruses |
| Persistent, productive | No cytopathic effect; little metabolic disturbance; cells continue to divide; may be loss of the special functions of some differentiated cells | Yes | Pestiviruses, arenaviruses, rabies virus, most retroviruses |
| Persistent, nonproductive | No effect or loss of specialized functions | No, but virus may be induced by trauma, etc. | Papillomavirus in the skin |
| Transformation       | Alteration in cell morphology; cells can be passaged indefinitely; may produce tumors when transplanted to experimental animals | Yes, oncogenic DNA viruses, oncogenic retroviruses | Polyomavirus, adenoviruses, Murine, avian leukosis and sarcoma viruses |
Cytopathic effects produced by viruses. Inclusions may reflect viral replication complexes in the nucleus or cytoplasm. Cell rounding may follow cytoskeletal disruption. Syncytia formation may be seen following infection with enveloped viruses. Apoptosis is a programmed cell death resulting in morphologic changes that are distinct from necrosis or lysis (i.e., forms of nonprogrammed cell death), and is a form of host defense. A single virus may cause combinations of these cytopathic effects. Courtesy of M. Oglesbee and S. Niewiesk, The Ohio State University.

Inhibition of Host Cell Protein Production while viral protein synthesis continues is a characteristic of many viral infections. This shutdown is particularly rapid and profound in picornavirus infections, but it is also pronounced in togavirus, influenza virus, rhabdovirus, poxvirus, and herpesvirus infections. With some other viruses, the shutdown occurs late in the course of infection and is more gradual, whereas with noncytoidal viruses, such as pestiviruses, arenaviruses, and retroviruses, there is no dramatic inhibition of host-cell protein synthesis, and no signal for protein, and blue signal for nucleic acid. Intranuclear inclusions typically stain red, indicative of the high viral protein content, whereas the marginalized chromatin is blue. Intranuclear inclusions are characteristic of cells infected with herpesviruses and adenoviruses. Occasionally an RNA virus will induce structures known as nuclear bodies, a type of intranuclear inclusion that is host in origin but rich in viral protein. These structures are thought to regulate RNA processing within the cell, and are the basis for the intranuclear inclusions of canine distemper virus infection. Cytoplasmic inclusions are typical of viruses replicating to high levels in the cytoplasm, again reflecting aggregates of viral genomes engaged in transcription and replication. Cytoplasmic inclusions are typical of infections caused by poxviruses, paramyxoviruses, rhabdoviruses, and reoviruses (Fig. 2.2). The diagnostic importance of these structures is illustrated by the fact that some of these inclusions are known by specific names, such as the “Negri bodies” of rabies virus infected neurons.

**Inhibition of Host Cell Protein Production** while viral protein synthesis continues is a characteristic of many viral infections. This shutdown is particularly rapid and profound in picornavirus infections, but it is also pronounced in togavirus, influenza virus, rhabdovirus, poxvirus, and herpesvirus infections. With some other viruses, the shutdown occurs late in the course of infection and is more gradual, whereas with noncytoidal viruses, such as pestiviruses, arenaviruses, and retroviruses, there is no dramatic inhibition of host-cell protein synthesis, and no cell death. Viruses have evolved numerous mechanisms to interfere with host-cell mRNA transcription, processing, and translation. Inhibition of both host-cell DNA replication and mRNA transcription is a consequence of DNA virus infection when cellular machinery is redirected to viral templates. Inhibition may reflect a broader strategy by the virus to preserve nucleotide pools in support of virus replication, and to diminish cellular mRNA levels that would otherwise compete with viral mRNA for translational machinery. This phenomenon is observed during replication of viruses in several different families, including poxviruses, rhabdoviruses, reoviruses, paramyxoviruses, and picornaviruses. In some instances, this inhibition may be the indirect consequence of viral effects on host-cell protein synthesis that decrease the availability of transcription factors required for DNA-dependent RNA polymerase activity.

Inhibition of processing and translation of host-cell messenger RNA occurs during replication of vesicular stomatitis viruses, influenza viruses, and herpesviruses, through interference with the splicing of cellular primary mRNA transcripts that are needed to form mature mRNAs. In some instances, spliceosomes are formed, but subsequent catalytic steps are inhibited. For example, a protein synthesized in herpesvirus-infected cells suppresses RNA splicing and leads to reduced amounts of cellular mRNAs and the accumulation of primary mRNA transcripts. In addition to interference with host-cell mRNA transcription and processing, viruses may produce factors that bind to ribosomes and inhibit cellular mRNA translation. Viral proteins may inhibit the processing and transport of cellular proteins from the endoplasmic reticulum, and this inhibition may lead to their degradation. This effect is seen in lentivirus and adenovirus infections. Influenza viruses remove the 5' cap structure of cellular mRNAs, the cap being required for translation. Other viruses simply produce viral mRNAs in large quantities in order to assure translation, outcompeting cellular mRNAs for cellular translation machinery by mass action.

The cumulative effect of inhibition of host-cell protein synthesis and depletion of nucleotide pools can be the loss of cellular homeostasis, resulting in a sequence of degeneration and necrosis. This progression is relatively nonspecific as to cause, with similar changes being induced by physical or chemical insults. The most common early and potentially reversible change is cloudy swelling, a change associated with increasing permeability of the cellular membranes leading to swelling of the nucleus, distention of the endoplasmic reticulum and mitochondria, and rarefaction of the cytoplasm. Later in the course of many viral infections the nucleus becomes condensed and shrunken, and cytoplasmic density increases. Cell destruction can be the consequence of
further loss of osmotic integrity and leakage of lysosomal enzymes into the cytoplasm. This progression is consistent with the so-called common terminal pathway to cell death. In contrast to these nonspecific changes are toxicities induced by viral proteins that interfere with cellular membrane or cytoskeletal structure and function.

**Interference with Cellular Membrane Function** can affect the participation of cellular membranes in many phases of virus replication, from virus attachment and entry, to the formation of replication complexes, to virion assembly. Viruses may alter plasma membrane permeability, affect ion exchange and membrane potential, or induce the synthesis of new intracellular membranes or the rearrangement of previously existing ones. For example, a generalized increase in membrane permeability occurs early during picornavirus, alphavirus, reovirus, rhabdovirus, and adenovirus infections. Early changes in cell structure often are dominated by proliferation of various cell membranes: for example, herpesviruses cause increased synthesis, even reduplication, of nuclear membranes; flaviviruses cause proliferation of the endoplasmic reticulum; picornaviruses and caliciviruses cause a distinctive proliferation of vesicles in the cytoplasm; and many retroviruses cause peculiar fusions of cytoplasmic membranes.

Enveloped viruses specifically direct the insertion of their surface glycoproteins, including fusion proteins, into host-cell membranes as part of their budding process, and this may lead to membrane fusion between infected and uninfected cells, resulting in the formation of a multinucleated syncytium. This activity is restricted to enveloped viruses whose fusion proteins are activated when viral membrane glycoproteins come in contact with a cellular receptor. Normally this process allows fusion of the virion envelope with the cytoplasmic membrane of a target cell during the initiation of an infection, allowing entry the viral genome into the cytoplasm. In the course of virus replication however, these same fusion proteins are inserted into the cytoplasmic membrane of the infected cell in preparation for viral budding (Fig. 3.10). If viral membrane glycoproteins engage receptors on neighboring cells, the fusion proteins may be activated to cause fusion of one cell membrane with another, giving rise to the syncytial cell. Syncytia are a conspicuous feature of infection of cell monolayers in culture by lentiviruses, coronaviruses, paramyxoviruses, and some herpesviruses. Syncytia may also be observed in tissue of infected animals, particularly for paramyxoviruses; for example, in horses infected with Hendra virus and cattle infected with respiratory syncytial virus. Syncytium formation has been suggested as a means by which viruses spread in tissues: fusion bridges may allow subviral entities, such as viral nucleocapsids and nucleic acids, to spread while avoiding host defenses. Relevance of this mechanism is limited to specific cell types, being implicated as a means for neuron-to-neuron spread of rhabdoviruses and paramyxoviruses where fusion events are likely restricted to very small cell contact points within the synapse.

Viral proteins (antigens) inserted into the host-cell plasma membrane may constitute targets for specific humoral and cellular immune responses that cause the lysis of the infected cell. This may happen before significant progeny virus is produced, thus slowing or arresting the progress of infection and hastening recovery (see Chapter 4: Antiviral Immunity and Virus Vaccines). It is for this reason that accumulation of viral membrane glycoproteins occurs late in the infection cycle, in preparation for viral budding. Although these viral proteins are attractive targets for immune clearance, the host response may also contribute to immune-mediated tissue injury and disease. Viral antigens may also be incorporated in the membrane of cells transformed by viruses, and play an important role in immune-mediated resolution or regression of viral papillomas, for example.

![FIGURE 3.10 Formation of syncytia by enveloped viruses. Following enveloped virus attachment and penetration, the genome is transcribed to produce the envelope proteins that are essential to these processes: attachment to and fusion between the viral envelope and the cell membrane. These viral proteins are inserted into the cell membrane in preparation for viral assembly (budding), but if they contact a neighboring uninfected cell, they can mediate attachment to and fusion with the membranes of that neighboring cell. The result is a multinucleated syncytium. Courtesy of M. Oglesbee and S. Niewiesk, The Ohio State University.](image)
The Intrinsic (Mitochondrial) Pathway

The Extrinsic (Death Receptor) Pathway

Hemadsorption and hemagglutination. Viral envelope proteins may bind glycoproteins expressed on the surface of erythrocytes from a species other than the natural host of the virus. Diagnostic tests have been developed that exploit this phenomenon. Erythrocytes may bind to infected cells that express these viral envelope proteins on their surface (hemadsorption) or cell free viruses may cross-link erythrocytes to form aggregates (hemagglutination), indicating the presence of virus infection. Courtesy of M. Oglesbee and S. Niewiesk, The Ohio State University.

FIGURE 3.11 Hemadsorption and hemagglutination. Viral envelope proteins may bind to glycoproteins expressed on the surface of erythrocytes from a species other than the natural host of the virus. Diagnostic tests have been developed that exploit this phenomenon. Erythrocytes may bind to infected cells that express these viral envelope proteins on their surface (hemadsorption) or cell free viruses may cross-link erythrocytes to form aggregates (hemagglutination), indicating the presence of virus infection. Courtesy of M. Oglesbee and S. Niewiesk, The Ohio State University.

surface of erythrocytes. The same glycoprotein spikes are responsible for hemagglutination in vitro—that is, the agglutination of erythrocytes by free viral particles. Hemadsorption and hemagglutination are not known to play a part in the pathogenesis of viral diseases.

Disruption of the Cell Cytoskeleton causes changes in cell shape (eg, rounding) that are characteristic of many viral infections. The cytoskeleton is made up of several filament systems, including microfilaments (eg, actin), intermediate filaments (eg, vimentin), and microtubules (eg, tubulin). The cytoskeleton is responsible for the structural integrity of the cell, for the transport of organelles through the cell, and for certain cell motility activities. Particular viruses may damage specific filament systems: for example, canine distemper virus, vesicular stomatitis viruses, vaccinia virus, and herpesviruses cause a depolymerization of actin-containing microfilaments, and enteroviruses induce extensive damage to microtubules. Such damage contributes to the drastic cytopathic changes that precede cell lysis in many infections. The elements of the cytoskeleton are also employed by many viruses in the course of their replication: in virus entry, in the formation of replication complexes and assembly sites, and in virion release.

Apoptosis is the process of programmed cell death, which is essentially a mechanism of cell suicide that the host activates as a last resort to eliminate viral factories before progeny virus production is complete. It was long thought that viruses killed cells exclusively by direct means such as usurping their cellular machinery or disrupting membrane integrity, ultimately leading to necrosis of the virus-infected cell. However, it is now clear that apoptosis is an important and common event during many viral infections. There are two distinct cellular pathways that trigger apoptosis, both of which culminate in the activation of host-cell caspase enzymes that mediate death of the cell (the so-called executioner phase). Once activated, caspases are responsible for degradation of the cell’s own DNA and proteins. Cell membrane alterations in the doomed cell promote its recognition and removal by phagocytic cells. The two initiation pathways are:

1. The Intrinsic (Mitochondrial) Pathway. The mitochondrial pathway is activated as a result of increased permeability of mitochondrial membranes subsequent to cell injury, such as that associated with a viral infection. Severe injury alters the delicate balance between antiapoptotic (eg, Bcl-2) and proapoptotic (eg, Bax) molecules in mitochondrial membranes and the cytosol, resulting in progressive leakage of mitochondrial proteins (such as cytochrome c) into the cytosol where these proteins activate cellular caspases.

2. The Extrinsic (Death Receptor) Pathway. The extrinsic pathway is activated by engagement of specific cell-membrane receptors, which are members of the tissue necrosis factor (TNF) receptor family (TNF, Fas, and others). Thus binding of tissue necrosis factor to its cellular receptor can trigger apoptosis. Similarly, cytotoxic T lymphocytes that recognize virus-infected cells in an antigen-specific manner can bind the Fas receptor, activate the death domain, and trigger the executioner caspase pathway that then eliminates the cell before it becomes a functional virus factory.

Noncytopathic Changes in Virus-Infected Cells

Noncytopathic viral infections usually do not kill the cells in which replication occurs. On the contrary, they often cause persistent infection during which infected cells produce and release virions but cellular metabolism that is essential to maintaining homeostasis is either not affected or is minimally affected. In many instances, infected cells even continue to grow and divide. This type of interaction can occur in cells infected with several kinds of RNA viruses, notably pestiviruses, arenaviruses, retroviruses, and some paramyxoviruses. Nevertheless, with few exceptions (eg, some retroviruses), there are slowly progressive changes that ultimately lead to cell death. In the host animal, cell replacement occurs so rapidly in most organs and tissues that the slow fallout of cells as a result of persistent infection may have no effect on overall function, whereas terminally differentiated cells such as neurons, once destroyed, are not replaced, and persistently infected differentiated cells may lose their capacity to carry out specialized functions.

Viruses such as the pestiviruses, arenaviruses, Bornavirus, and retroviruses that do not shut down host-cell protein, RNA, or DNA synthesis and that do not rapidly kill their host cells, can produce important
pathophysiologic changes in their hosts by affecting crucial functions that are associated neither with the integrity of cells nor their basic housekeeping functions. Damage to the specialized functions of differentiated cells may still affect complex regulatory, homeostatic, and metabolic functions, including those of the central nervous system, endocrine glands, and immune system.

**Virus-Mediated Tissue and Organ Injury**

The severity of a viral disease is not necessarily correlated with the degree of cytopathology produced by the causative virus in cells in culture. Many viruses that are cytocidal in cultured cells do not produce clinical signs in vivo (eg, many enteroviruses), whereas some that are noncytocidal in vitro cause lethal disease in animals (eg, rabies virus). Further, depending on the organ affected, cell and tissue damage can occur without producing clinical signs of disease—for example, a large number of hepatocytes (liver cells) may be destroyed in Rift Valley fever in sheep without obvious clinical signs. When damage to cells does impair the function of an organ or tissue, this may be relatively insignificant in a tissue such as skeletal muscle, but potentially devastating in organs such as the heart or the brain. Likewise, virus-induced inflammation and edema are especially serious consequences in organs such as the lungs and CNS.

**Mechanisms of Viral Infection and Injury of Target Tissues and Organs**

The mechanisms by which individual viruses cause injury to their specific target organs are described in detail under individual virus families in Part II of this book, thus the objective of this section is to provide a brief overview of potential pathogenic mechanisms that viruses can use to cause injury in their target tissues.

**Viral Infection of the Respiratory Tract**

Viral infections of the respiratory tract are extremely common, especially in animals housed in crowded settings. Individual viruses exhibit tropism for different levels of the respiratory tract, from the nasal passages to the pulmonary airspaces (terminal airways and alveoli), but there is considerable overlap. Tropism of respiratory viruses is probably a reflection of the distribution of appropriate receptors and intracellular transcriptional enhancers, as well as physical barriers, physiological factors, and immune parameters. For example, bovine rhinitis viruses (Family Picornaviridae) replicate in the nasal passages because their replication is optimized at lower temperatures, whereas bovine respiratory syncytial virus (Family Paramyxoviridae) preferentially infects epithelial cells lining the terminal airways; thus rhinitis viruses may cause mild rhinitis, whereas respiratory syncytial virus is the cause of bronchiolitis and bronchointerstitial pneumonia. Some viruses cause injury to the type I or type II pneumocytes lining the alveoli, either directly or indirectly; if extensive, injury to type I pneumocytes leads to acute respiratory distress syndrome, whereas injury to type II pneumocytes delays repair and healing in the affected lung.

Influenza viruses replicate in both the nasal passages and airways of infected mammals, but influenza virus infection is typically confined to the lung because of the requirement for hemagglutinin cleavage by tissue-specific proteases. However, highly virulent influenza viruses such as the Eurasian—African H5N1 virus can spread beyond the lungs to cause severe generalized (systemic) infection and disease. The ability of this virus to escape the lung may be related to its tropism for type I pneumocytes that line alveoli, and its ability to cause systemic disease may reflect the fact that its hemagglutinin can be cleaved by proteases that are present in many tissues. Similarly in birds, high-pathogenicity avian influenza viruses have several basic amino acids at the hemagglutinin cleavage site, which can be cleaved intracellularly by ubiquitous endopeptidase furins located in the trans-Golgi network in a wide variety of cell types in various tissues. In contrast, the hemagglutinin protein of low pathogenicity avian influenza viruses is cleaved extracellularly by tissue-restricted proteases that are confined to the respiratory and gastrointestinal tracts (see Chapter 21: Orthomyxoviridae).

Regardless of the level of the respiratory tree that is initially infected, viral infection typically leads to local cessation of ciliary activity, focal loss of integrity of the lining mucus layer, and multifocal destruction of small numbers of epithelial cells (Fig. 3.12). Initial injury is followed by progressive infection of epithelial cells within the mucosa, and inflammation of increasing severity, with exudation of fluid and influx of inflammatory cells. Fibrin-rich inflammatory exudate and necrotic cellular debris (degenerate neutrophils and sloughed epithelium) then accumulate in the lumen of the affected airways or passages, with subsequent obstruction and, in severe cases, increasing hypoxia and respiratory distress. The mucosa is quickly regenerated in animals that survive, and adaptive immune responses clear the infecting virus and prevent reinfection for variable periods of time (depending on the particular virus).

In addition to their direct adverse consequences, viral infections of the respiratory tract often predispose animals to secondary infections with bacteria, even those bacteria that constitute the normal flora in the nose and throat. This predisposition can result from interference with normal mucociliary clearance as a consequence of viral injury to the mucosa, or suppression of innate immune
responses. For example, cellular expression of Toll-like receptors is depressed in the lung after influenza virus infection, and thus convalescent animals may be less able to quickly recognize and neutralize invading bacteria. This potential synergy between respiratory viruses and bacteria is compounded by overcrowding of animals as occurs during shipping and in feedlots and shelters. Environmental factors may combine to facilitate concurrent airway infection by multiple viruses and bacteria. These polymicrobial infections are facilitated by the immunosuppressive effects of stress, the induction of ciliary stasis by exposure to ammonia vapor from animal waste, and crowded humid environments that facilitate aerosol transmission of enveloped viruses.

Viral Infection of the Gastrointestinal Tract

Infection of the gastrointestinal tract can be acquired either by ingestion of an enteric virus (e.g., rotaviruses, coronaviruses, astroviruses, etc.) where infection is confined to the gastrointestinal tract or as a consequence of generalized hematogenous spread during a systemic viral infection such as with certain parvoviruses (e.g., feline panleukopenia, canine parvovirus), pestiviruses (e.g., bovine viral diarrhea virus), and morbilliviruses (e.g., canine distemper and rinderpest viruses). Enteric viral infections usually result in rapid onset of gastrointestinal disease after a short incubation period, whereas systemic infections have a longer incubation period and are typically accompanied by clinical signs that are not confined to dysfunction of the gastrointestinal tract.

Virus-induced diarrhea is a result of infection of the epithelial cells (enterocytes) lining the gastrointestinal mucosa. Rotaviruses, astroviruses, and enteric coronaviruses characteristically infect the more mature enterocytes that line the intestinal villi, whereas parvoviruses and pestiviruses infect and destroy the immature and dividing enterocytes present in the intestinal crypts. Regardless of their site of predilection, these infections all destroy enterocytes in the gastrointestinal mucosa and so reduce its absorptive surface, leading to malabsorption diarrhea with attendant loss of both fluid and electrolytes. The pathogenesis of enteric virus infections can be even more complex than simple virus-mediated destruction of enterocytes; for example, rotaviruses produce a protein (nsp4) that itself causes secretion of fluid into the bowel (intestinal hypersecretion), even in the absence of substantial virus-mediated damage. In suckling neonates, undigested lactose from ingested milk passes through the small bowel to the large bowel, where it exerts an osmotic effect that further exacerbates fluid loss. Neonates are also disadvantaged by the fact that the replacement rate of enterocytes is not as high as in older animals. Animals with severe diarrhea can rapidly develop pronounced dehydration, hemoconcentration, acidosis that inhibits critical enzymes and metabolic pathways, hypoglycemia, and systemic electrolyte disturbances (typically, decreased sodium and increased potassium), and diarrhea can be quickly fatal in very young or otherwise compromised animals.

Enteric virus infections that occur via the oral route generally begin in the stomach or proximal small intestine, and they then spread caudally as a “wave” that sequentially affects the jejunum, ileum, and large bowel. As the infection progresses through the bowel, absorptive cells destroyed by the infecting virus are quickly replaced by immature enterocytes from the intestinal crypts. The presence of increased numbers of these immature enterocytes contributes to malabsorption and intestinal hypersecretion (fluid and electrolyte loss). Adaptive immune responses lead to mucosal IgA and systemic IgG production in animals that survive, conferring resistance to reinfection. Enteric virus infections in neonates are frequently associated with infections by other enteric pathogens, including bacteria (e.g., enterotoxigenic or enteropathogenic Escherichia coli) and protozoa such as Cryptosporidium spp., probably because of the common

![Figure 3.12](image-url)
factors (crowding, poor sanitation) that predispose to these infections.

Viral Infection of the Skin

In addition to being a site of initial infection, the skin may be invaded secondarily via the bloodstream. Thus skin lesions that accompany viral infections can be either localized, such as papillomas, or disseminated. In animals, erythema (reddening) of the skin as a consequence of systemic viral infections is most obvious on exposed, hairless, nonpigmented areas such as the snout, ears, paws, scrotum, and udder. In addition to papillomas (warts, see Chapter 11: Papillomaviridae and Polyomaviridae), virus-induced lesions that commonly affect the skin of virus-infected animals include macules (flat discolored areas of skin), papules (raised areas of skin), vesicles (fluid-filled raised areas of skin), and pustules (raised areas of skin containing leukocytes). Viruses of particular families tend to produce characteristic cutaneous lesions, frequently in association with similar lesions in the oral and nasal mucosa, the teats and genitalia, and at the junction of the hooves and skin of ungulates. Vesicles are especially important cutaneous lesions that are characteristic of important, potentially reportable diseases of livestock; in particular, vesicle formation is characteristic of foot-and-mouth disease and other viral diseases that can mimic it, although vesicles clearly can occur in other diseases not caused by viruses. Vesicles are essentially discrete “blisters” that result from accumulation of edema fluid within the affected epidermis, or separation of the epidermis from the underlying dermis (or mucosal epithelium from the submucosa). Vesicles rupture quickly to leave focal ulcers. Papules are either localized (eg, orf) or disseminated (eg, lumpy skin disease) epithelial proliferations that are characteristic of poxvirus infections. These proliferative and raised lesions frequently become extensively encrusted with inflammatory exudate.

Virus infections that result in widespread endothelial injury in blood vessels throughout the body, including those of the subcutaneous tissues, can produce subcutaneous edema and erythema or hemorrhages in the skin and elsewhere (including the oral cavity and internal organs).

Viral Infection of the Central Nervous System

The CNS (brain and spinal cord) is exquisitely susceptible to serious, often fatal injury by certain viral infections provided the virus can gain access to these tissues. Viruses can spread from distal sites to the brain via nerves (Fig. 3.7), or via the blood (Fig. 3.13). Spread via nerves may involve peripheral nerve endings or infection of olfactory neurons in the nasal cavity with subsequent viral transport by axons of the olfactory nerve directly into the brain. To spread from the blood, viruses must cross either the blood–brain or blood–cerebrospinal fluid barriers. The blood–brain barrier consists of endothelial cells that are nonfenestrated and connected by tight junctions, which in turn are surrounded by a basement membrane and astrocytes. Viruses may cross this barrier by either direct infection of endothelial cells and spread of infection to the adjacent astrocytes, or the virus may be carried across the barrier by infected leukocytes that are engaged in immune surveillance of the CNS or inflammatory responses. The blood–cerebrospinal fluid barrier is formed by tight junctions between epithelial cells of the choroid plexus, which are highly vascular structures producing the cerebrospinal fluids that circulate within the

![FIGURE 3.13](image-url) Routes of viral invasion of the central nervous system. Virus may reach the brain via the blood vasculature, crossing the blood–brain barrier (BBB) or the blood–cerebrospinal fluid (CSF) barrier. The BBB is formed by capillary lining cells that lack pores and are tightly bound together, and are surrounded by processes of astrocytes (top inset). Viruses cross the BBB by infecting the capillary lining cells and then infecting the astrocytes, or the virus is carried across the barrier within infected leukocytes. To cross the blood–CSF barrier, virus infects epithelial cells of the choroid plexus, the structures responsible for producing CSF that circulates in the ventricular network of the brain. Capillaries in the plexus are leaky, allowing virus ready access to the plexus epithelial cells. Infected epithelial cells can then shed virus into CSF. Neural spread of virus can occur by infecting olfactory neurons in the nasal cavity, using axon transporters and trans-synaptic spread to carry the virus infection into the brain. Courtesy of M. Oglesbee and S. Niewiesk, The Ohio State University.
ventricles of the brain, the central canal of the spinal cord, and the leptomeninges that cover the surface of both brain and spinal cord. There is no barrier between the bloodstream and the epithelial cells of the plexus, and if virus can infect the choroid plexus epithelial cells, it may be shed through the cerebrospinal fluid pathways to be widely disseminated in the CNS. Collectively, a virus’ ability to overcome these barriers and initiate CNS infection are known as neuroinvasiveness.

Once present within the CNS, a number of viruses can quickly spread to cause progressive infection of neurons and/or glial cells (astrocytes, microglia, and oligodendrocytes). This capability is known as neurovirulence. A virus can be poorly neuroinvasive, but if infection is initiated, exhibit a high degree of neurovirulence. Cytopathic infections of neurons, whether caused by togaviruses, flaviviruses, herpesviruses, or other viruses, leads to encephalitis or encephalomyelitis characterized by neuronal necrosis, phagocytosis of neurons (neuronophagia), and perivascular infiltrations of inflammatory cells (perivascular cuffing). The small vessels of the meninges are frequently involved in virus-induced inflammation of the CNS, either alone (meningitis) or in combination with inflammation of the brain and spinal cord (meningoencephalitis and meningomyelitis). In contrast, virulent rabies virus infection of neurons is noncytocidal and evokes little inflammatory reaction, but it is uniformly lethal for most mammalian species.

Other characteristic pathologic changes are produced by various viruses, and by prions that cause slowly progressive diseases of the CNS. In bovine spongiform encephalopathy in cattle and scrapie in sheep, for example, there is slowly progressive neuronal degeneration and vacuolization. In contrast, infection of glial cells in dogs with canine distemper leads to progressive infection of neurons and/or glial cells (astrocytes, microglia, and oligodendrocytes). This capability is known as neurovirulence. A virus can be poorly neuroinvasive, but if infection is initiated, exhibit a high degree of neurovirulence. Cytopathic infections of neurons, whether caused by togaviruses, flaviviruses, herpesviruses, or other viruses, leads to encephalitis or encephalomyelitis characterized by neuronal necrosis, phagocytosis of neurons (neuronophagia), and perivascular infiltrations of inflammatory cells (perivascular cuffing). The small vessels of the meninges are frequently involved in virus-induced inflammation of the CNS, either alone (meningitis) or in combination with inflammation of the brain and spinal cord (meningoencephalitis and meningomyelitis). In contrast, virulent rabies virus infection of neurons is noncytocidal and evokes little inflammatory reaction, but it is uniformly lethal for most mammalian species.

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In most cases, infection of the CNS seems to be a dead end in the natural history of viruses—shedding and transmission does not occur, particularly when the infection is highly cytopathic. Viruses that successfully use neurons for transmission are in the minority and they typically exhibit noncytopathic or poorly cytopathic infections. Noncytopathic infection of neurons is needed for rabies virus to complete the cycle of infection within a host. Rabies is reliant upon axon transporters of viable cells to travel from the point of inoculation to the CNS, to spread within the CNS, and to spread from the CNS to peripheral organs such as salivary glands that amplify and shed progeny virus. Cell death and the attending inflammatory response can prevent the virus from completing this cycle in more highly cytopathic infections, where the virus is less adapted to its host. The alphaherpesviruses undergo latent infection of peripheral nerves, specifically the dorsal root ganglion neurons. When reactivated, the infection is productive yet noncytopathic for the neuron. Progeny infect epithelial cells of mucosal surfaces where the infection is both productive and cytopathic. All in all, it seems anomalous that neurotropism should be the outstanding characteristic of so many of the most notorious pathogens of animals and zoonotic pathogens of humans, and yet be the characteristic least related to virus propagation in nature.

**Viral Infection of the Hemopoietic System**

The hemopoietic system includes: (1) the myeloid tissues, specifically the bone marrow and cells derived from it—erythrocytes, platelets, monocytes, and granulocytes; and (2) the lymphoid tissues, which include the thymus, lymph nodes, spleen, mucosa-associated lymphoid tissues and, in birds, the cloacal bursa. Cells that populate the myeloid and lymphoid systems, including lymphocytes, dendritic cells, and cells of the mononuclear phagocytic system (monocytes and macrophages) are all derived from bone marrow (or equivalent hemopoietic tissue) precursors. It is therefore convenient to group these cells and tissues under the heading of the hemopoietic system and to dispense with historical terminology such as “lymphoreticular” or “reticuloendothelial” systems. Importantly, lymphocytes and mononuclear phagocytes (blood monocytes, tissue macrophages, dendritic cells) are responsible for adaptive immunity (see Chapter 4: Antiviral Immunity and Virus Vaccines), thus viral infections of these cells can have profound effects on immunity.

Infection and damage to mononuclear phagocytes can inhibit both the innate and adaptive immune response to the virus, in addition to serving as a source of progeny virions. Some of the most destructive and lethal viruses known exhibit this tropism: filoviruses, arenaviruses, hantaviruses, orbiviruses such as African horse sickness and bluetongue viruses, certain bunyaviruses such as Rift Valley fever virus, alphaviruses such as Venezuelan equine encephalitis virus, and flaviviruses such as yellow fever virus. After initial invasion, infection with these viruses begins with their uptake by dendritic cells and/or macrophages in lymphoid tissues (lymph nodes, thymus, bone marrow, Peyer’s patches, and the white pulp of the spleen). Viral infection can then spread in these tissues, frequently leading to cytolysis of adjacent lymphocytes and immune dysfunction.

Viral infections can result in either specific acquired immunodeficiency or generalized immunosuppression. A relevant example of this phenomenon is provided by infection of the cloacal bursa (bursa of Fabricius) in chickens (the site of B cell differentiation in birds) with infectious bursal disease virus, which leads to atrophy of the bursa and a severe deficiency of B lymphocytes,
equivalent to bursectomy. The result is an inability of severely affected birds to develop antibody-mediated immune responses to other infectious agents, which in turn leads to an increase in susceptibility to bacterial infections such as those caused by Salmonella spp. and E. coli, and other viruses. Acquired immunodeficiency syndrome (AIDS) in humans is caused by the human immunodeficiency virus (HIV), and similar viruses infect monkeys (simian immunodeficiency viruses), cattle (bovine immunodeficiency virus), and cats (feline immunodeficiency virus). In susceptible animals, these viruses individually can infect and destroy specific but different cells of the immune system, thereby causing immunosuppression of different types and severity.

Many other viruses (eg, classical swine fever virus, bovine viral diarrhea virus, canine distemper virus, feline and canine parvoviruses) that cause systemic infections, especially those that infect mononuclear phagocytes and/or lymphocytes, may temporarily but globally suppress adaptive immune responses, both humoral and cell-mediated. Affected animals are predisposed to diseases caused by other infectious agents during the period of virus-induced immunosuppression, a phenomenon that can also occur following vaccination with certain live-attenuated vaccines. The immune response to unrelated antigens may be reduced or abrogated in animals undergoing such infections.

Virus-induced immunosuppression may in turn lead to enhanced virus replication, such as the reactivation of latent herpesvirus, adenovirus, or polyomavirus infections. Similarly, immunosuppression associated with administration of cytotoxic drugs or irradiation for chemotherapy or organ transplantation can predispose to recrudescence of herpesviruses and, potentially, others.

Viral Infection of the Fetus

Most viral infections of the dam do not lead to infection of the fetus due to barrier functions provided by the placenta, although severe infections of the dam can sometimes lead to fetal death and expulsion (abortion) in the absence of fetal infection. However, some viruses can cross the placenta to infect the fetus (Table 3.3). Such infections occur most commonly in young dams (such as first-calf heifers) that are exposed during pregnancy to pathogenic viruses to which they have no immunity, as a consequence of lack of either appropriate vaccination or previous natural infection. The outcome of fetal viral infection is dependent upon the properties (virulence and tropism) of the infecting virus, as well as the gestational age of the fetus at the time of infection. Severe cytolytic infections of the fetus, especially in early gestation, are likely to cause fetal death and resorption or abortion, which also is dependent on the species of animal affected—abortion is especially common in those species in which pregnancy is sustained by fetal production of progesterone (such as sheep), whereas pregnancy is less likely to be terminated prematurely in multiparous species in which pregnancy is maintained by maternally derived progesterone (such as swine).

Teratogenic viruses are those that can cause developmental defects after in utero infection. The outcome of infections of pregnant animals with teratogenic viruses is influenced to a great extent by gestational age, which influences stages of organogenesis, degree to which biological barriers have formed in tissues such as the CNS, and degree of immune competence. Viral infections that occur during critical stages of organogenesis in the developing fetus can have devastating consequences from virus-mediated infection and destruction of progenitor cells before they can populate organs such as the brain. For example, Akabane, Cache Valley and Schmallenberg viruses, bovine viral diarrhea virus, and bluetongue virus can all cause teratogenic brain defects in congenitally infected ruminants, as can parvovirus infections in cats.

Although immune competence generally is developed by mid-gestation, viral infections before this time can lead to a weak and ineffectual immune response that leads to persistent postnatal infection, such as persistent bovine viral diarrhea virus infection in cattle and congenital lymphocytic choriomeningitis virus infection in mice.

Viral Infection of Other Organs

Almost any organ may be infected with one or another kind of virus via the bloodstream, but most viruses have well-defined organ and tissue tropisms that reflect the factors described earlier (presence of receptors, intracellular and other physiological or physical determinants of infection). The clinical importance of infection of various organs and tissues depends, in part, on their role in the physiologic well-being of the animal. In addition to the organs and tissues already described (respiratory tract, gastrointestinal tract, skin, brain and spinal cord, hematopoietic tissues), viral infections of the heart and liver can also have especially devastating consequences. The liver is the target of relatively few viral infections of animals, in marked contrast to the numerous hepatitis viruses (hepatitis A, B, and C viruses in particular) and other viruses (eg, yellow fever virus) that are important causes of severe liver disease in humans. In animals, Rift Valley fever virus, mouse hepatitis virus, and infectious canine hepatitis virus characteristically affect the liver, as do several abortogenic herpesviruses after fetal infections (eg, infectious bovine rhinotracheitis virus, equine herpesvirus 1, pseudorabies virus). Virus-mediated cardiac injury is relatively uncommon in animals, but is characteristic of
bluetongue and some other endotheliotrophic viral infections, and alphavirus infections of Atlantic salmon and rainbow trout.

Viruses that cause widespread vascular injury can result in disseminated hemorrhages and/or edema as a result of increased vascular permeability. Vascular injury in these so-called hemorrhagic viral fevers can result either from viral infection of endothelial cells or the systemic release of vasoactive and inflammatory mediators such as tissue necrosis factor from other infected cells—particularly mononuclear phagocytes and dendritic cells. Viruses causing vascular injury include dengue virus, yellow fever virus, ebola virus, and different hantavirus infections in humans, and bluetongue and African horse sickness viruses in livestock. Widespread endothelial injury leads to thrombosis that may precipitate disseminated intravascular coagulation, which is the common pathway that leads to death of animals and humans.

### TABLE 3.3 Examples of Viral Infections of the Fetus or Embryo

| Animal | Family/Genus | Virus | Syndrome |
|--------|--------------|-------|----------|
| Cattle | Herpesviridae/Varicellovirus | Infectious bovine rhinotracheitis virus | Fetal death, abortion |
| | Retroviridae/Deltaretrovirus | Bovine leukemia virus | Inapparent infection, leukemia |
| | Reoviridae/Orbivirus | Bluetongue virus | Fetal death, abortion, congenital defects |
| | Bunyaviridae/Orthobunyavirus | Akabane and Schmallenberg viruses | Fetal death, abortion, stillbirth, congenital defects |
| | Flaviridae/Pestivirus | Bovine viral diarrhea virus | Fetal death, abortion, congenital defects, inapparent infection with lifelong carrier status and shedding |
| Horses | Herpesviridae/Varicellovirus | Equine herpesvirus 1 | Fetal death, abortion, neonatal disease |
| | Arteriviridae/Arterivirus | Equine arteritis virus | Fetal death, abortion |
| Swine | Herpesviridae/Varicellovirus | Pseudorabies virus | Fetal death, abortion |
| | Paroviridae/Parovirus | Swine parovirus | Fetal death, abortion, mummification, stillbirth, infertility |
| | Flaviridae/Flavivirus | Japanese encephalitis virus | Fetal death, abortion |
| | Flaviridae/Pestivirus | Classical swine fever (hog cholera) virus | Fetal death, abortion, congenital defects, inapparent infection with lifelong carrier status and shedding |
| Sheep | Reoviridae/Orbivirus | Bluetongue virus | Fetal death, abortion, congenital defects |
| | Bunyaviridae/Orthobunyavirus | Akabane, Cache Valley, and Schmallenberg viruses | Stillbirth, congenital defects |
| | Bunyaviridae/Phlebovirus | Rift Valley fever virus | Fetal death, abortion |
| | Bunyaviridae/Nairovirus | Nairobi sheep disease virus | Fetal death, abortion |
| | Flaviridae/Pestivirus | Border disease virus | Congenital defects |
| Dogs | Herpesviridae/Varicellovirus | Canine herpesvirus | Perinatal death |
| Cats | Paroviridae/Parovirus | Feline panleukopenia virus | Cerebellar hypoplasia |
| | Retroviridae/Gammaretrovirus | Feline leukemia virus | Inapparent infection, leukemia, fetal death |
| Mice | Paroviridae/Parovirus | Rat virus | Fetal death |
| | Arenaviridae/Arenavirus | Lymphocytic choriomeningitis virus | Inapparent infection, with lifelong carrier status and shedding |
| Chicken | Picornaviridae/Enterovirus | Avian encephalomyelitis virus | Congenital defects, fetal death |
| | Retroviridae/Alpharetrovirus | Avian leukemia/sarcoma viruses | Inapparent infection, leukemia, other diseases |
infected with a variety of viruses that directly or indirectly cause vascular injury. Paradoxically, these infected individuals bleed profusely due to the consumption of clotting factors.

**Nonspecific Pathophysiological Changes in Viral Diseases**

Some of the adverse consequences of viral infections cannot be attributed to direct cell destruction by the virus, to immunopathology, or other physiological responses that may include release of endogenous adrenal glucocorticoids in response to the stress of the infection. Viral diseases are accompanied frequently by a number of vague general clinical signs, such as fever, malaise, anorexia, and lassitude. Cytokines (interleukin-1 in particular) produced in the course of innate immune responses to infection may be responsible for some of these signs, which collectively can significantly reduce the animal’s performance. Less characterized are the potential neuropsychiatric effects of persistent viral infection of particular neuronal tracts, such as that caused by Borna disease virus. Borna disease virus infection is not lytic in neurons, but induces bizarre changes in the behavior of rats, cats, and horses.

**Virus-Induced Immunopathology** The adaptive immune response (eg, antibodies and cytotoxic T cells) to viruses could theoretically be harmful if the elimination of virus-infected cells leads to dangerous physiological consequences (eg, damage to liver or heart). The concept of virus-induced immunopathology is based on experimental results obtained in mouse models. Antibody-mediated immunopathology (also called type III hypersensitivity reactions) is caused by deposition of complexes of antigen and antibody (immune complexes) that initiate inflammation and tissue damage. Immune complexes circulate in blood in the course of most viral infections. The fate of the immune complexes depends on the ratio of antibody to antigen. The virus is typically cleared by tissue macrophages in infections where there is a large excess of antibody as compared with circulating virus, or even if there are equivalent amounts of antibody and virus. However, in some persistent infections, viral proteins (antigens) and/or virions are released continuously into the blood but the antibody response is weak and antibodies are of low avidity. In these instances, immune complexes are deposited in small blood vessels that function as filters, especially those of the renal glomeruli. Immune complexes continue to be deposited in glomeruli over periods of weeks, months, or even years, leading to their accumulation and subsequent immune-complex mediated glomerulonephritis. This phenomenon is observed in Aleutian mink disease (parvovirus infection), feline leukemia, and equine infectious anemia. A similar pathogenesis may underlie the progression of feline infectious peritonitis, a multisystemic disease associated with coronavirus infection in cats. T cell-mediated immunopathology (also called type IV reactions or delayed hypersensitivity reactions) has only been unequivocally demonstrated in mouse models of lymphocytic choriomeningitis virus infection.

**Viruses and Autoimmune Disease**

It has been proposed, with little definitive evidence, that viral infections may be responsible for autoimmune diseases in animals and humans. Proposed mechanisms for this largely hypothetical phenomenon focus on either unregulated or misdirected immune responses precipitated by a viral infection, or the presence of shared or equivalent antigens on infectious agents and host cells (molecular mimicry). Molecular mimicry clearly is responsible for immune-mediated diseases initiated by microbial infection, as classically illustrated by rheumatic heart disease in humans that is initiated by group A *Streptococcus* infection. In viruses, individual epitopes have been identified in several viruses that are also present in animal tissue, such as muscle or nervous tissue (eg, myelin basic protein). The antibodies to these epitopes might contribute to immune-mediated tissue damage during the course of viral infection, but their pathogenic role, if any, in initiating and potentiating autoimmune disease remains uncertain.

**Persistent Infection and Chronic Damage to Tissues and Organs**

Persistent infections of one type or another are produced by a wide range of viruses, and are common in veterinary medicine. Apart from enteric and respiratory viruses that cause transient infections that remain localized to their respective target organs, most other categories of viral infections include examples of persistent infection. Foot-and-mouth disease, for example, usually is an acute, self-limiting infection, but a carrier state of uncertain epidemiological relevance occurs in which virus persists in the oropharynx of a very few convalescent animals. In other instances, such as those associated with immunodeficiency viral infections, persistent viral infections lead to chronic diseases, even when the acute manifestations of infection have been trivial or subclinical. Finally, persistent infections can lead to continuing tissue injury, often with an immune-mediated basis.

Persistent viral infections are important for several reasons. For example, they may be reactivated and cause recrudescence episodes of disease in the individual host, or they may lead to immune-mediated disease or to neoplasia. Persistent infection may allow survival of a particular
virus in individual animals and herds, even after vaccination. Similarly, persistent infections may be of epidemiologic importance—the source of contagion in long-distance virus transport and in reintroduction after elimination of virus from a given herd, flock, region, or country.

Persistent infections are manifest in several ways. There are persistent infections in which virus is demonstrable continuously, whether or not there is ongoing disease. Disease may develop late, often with an immunological or neoplastic basis. In other instances, disease is not manifest in persistently infected animals; for example, in the deer mouse (Peromyscus maniculatus), the reservoir rodent host of Sin Nombre virus, and the etiologic agent of hantavirus pulmonary syndrome in humans, virus is shed in urine, saliva, and feces probably for the life of the animal, even in the face of neutralizing antibody.

A striking proportion of persistent infections involve the CNS. Restrictions in antigen presentation by neurons and glia and the activity of regulatory T cells combine to tightly regulate immune responses in the CNS. This regulation is important in order to assure that immune and inflammatory responses do not disrupt the highly specialized functions of terminally differentiated neurons and myelin producing cells. Myelin also contains unique antigens capable of eliciting autoimmune reactions, further emphasizing the importance of regulating immune responses in the CNS. This environment poses an excellent opportunity for a virus to avoid immune surveillance, and neurons and glia often exhibit limited permissiveness to virus gene expression that favors a noncytopathic persistent infection.

Latent infections are a form of persistence in which infectious virus is not demonstrable except when reactivation occurs. For example, in infectious pustular vulvovaginitis, the sexually transmitted disease caused in cattle by bovine herpesvirus 1, virus usually cannot be isolated from the latently infected cow except when there are recrudescence lesions. Viral latency may be maintained by restricted expression of genes that have the capacity to kill the cell. During latency, herpesviruses express only a few genes that are necessary in the maintenance of latency, notably so-called latency-associated transcripts. During reactivation, which is often stimulated by immunosuppression and/or by the action of a cytokine or hormone, the whole viral genome is transcribed again. This strategy protects the virus during its latent state from all host immune actions that would normally result in virus clearance.

The dynamic nature of virus—cell or virus—tissue interactions gives rise to a spectrum of clinical manifestations of disease associated with viral persistence (Fig. 3.14). Slow infections is a clinical term used to describe a slowly progressive disease, where the initiation of infection is subclinical, and evidence of disease builds slowly as the virus persists. Persistence is associated with a progressive increase in viral burden and antigen expression and the associated inflammatory and immune responses that are the basis for disease. An example of a slow virus infection is ovine progressive pneumonia caused by retrovirus infection (see Chapter 14: Retroviridae). In chronic diseases, there may be evidence of the initiation of infection (ie, the acute clinical episode) followed by the clinical manifestations of persistence in which disease progresses more rapidly following an incubation period. Canine distemper virus infection in the CNS can be manifest as a chronic disease following acute multisystemic infection, although the initial infection may go unrecognized and the incubation period for the appearance of clinical neurological disease may be prolonged as a slow virus infection. Viruses that undergo latency with episodes of periodic reactivation may similarly be manifest as chronic diseases. These examples highlight the limitations of using clinical terminology to describe virus infection of a host, where the presence or absence of the virus or the different types of virus—tissue interactions are considered separately. To further illustrate this latter point are examples where acute infections have late clinical manifestations in which continuing replication of the causative virus is not involved in the progression of the disease. For example, in the cerebellar hypoplasia syndrome that occurs in young cats as a result of fetal infection with feline panleukopenia virus, virus cannot be isolated at the time neurologic damage is diagnosed. In fact, because of this, the cerebellar syndrome was for many years considered to be an inherited malformation. Further, some persistent infections possess features of more than one of these categories. For example, all retrovirus infections are persistent and most exhibit features of latency, but the diseases they cause may be delayed following infection or only manifest as slowly progressive diseases.

Individual viruses employ a remarkable variety of strategies for successful evasion of host immune and inflammatory responses in vivo. These mechanisms include noncytocidal infections without expression of immunogenic proteins, replication in cells of the immune system or subversion of host innate and adaptive immunity (see Chapter 4: Antiviral Immunity and Virus Vaccines), and infection of nonpermissive, resting, or undifferentiated cells. Some viruses have evolved strategies for evading neutralization by the antibody they elicit. Ebola virus, for example, uses an “immune decoy” to evade neutralizing antibody—specifically, a secreted viral protein that binds circulating antibody. The surface glycoproteins of filoviruses, arenaviruses, bunyaviruses (eg, Rift Valley fever virus), and some arteriviruses
(eg, porcine reproductive and respiratory syndrome virus and lactate dehydrogenase-elevating virus) are heavily glycosylated, which may serve to mask the neutralizing epitopes contained in these proteins. Antigenic drift is especially characteristic of persistent RNA viral infection, particularly for lentiviruses (eg, equine infectious anemia virus). During persistent infection, sequential antigenic variants are produced, with each successive variant sufficiently different to evade the immune response raised against the preceding variant. In equine infectious anemia, clinical signs occur in periodic cycles, with each cycle being initiated by the emergence of a new viral variant. In addition to providing a mechanism for escape from immune elimination, each new variant may be more virulent than its predecessor, and this may directly affect the severity and progression of the disease.

The integration of retroviral proviral DNA into the genome of the host germ-line cells assures indefinite maintenance from one generation to the next; such proviral DNA can also lead to induction of tumors (oncogenesis).

VIRUS-INDUCED NEOPLASIA

Neoplasms arise as a consequence of the dysregulated growth of cells derived from a few or a single, genetically altered progenitor cell(s). Thus, although neoplasms are often composed of several cell types, they are considered to originate from an oligoclonal or monoclonal outgrowth of a single cell. The genetic changes that are ultimately responsible for neoplasia may be caused by naturally occurring mutations, chemical or physical agents or infectious agents including viruses, but all involve certain common cellular pathways.

The discoveries of the viral etiology of avian leukemia by Ellerman and Bang and of avian sarcoma by Rous, in 1908 and 1911, respectively, were long regarded as curiosities unlikely to be of any fundamental significance. However, study of these avian viruses and related retroviruses of mice has increased our overall understanding of neoplasia greatly, and since the 1950s there has been a steady stream of discoveries clearly incriminating other viruses in a variety of benign and malignant neoplasms of
The Cellular Basis of Neoplasia

Neoplasia is the result of nonlethal genetic injury, as may be acquired by chemical or physical damage, or from viral infections. Some cancers, however, arise randomly through the accumulation of spontaneous genetic mutations. A neoplasm results from the clonal expansion of cells that have suffered genetic damage, typically in one of four types of normal regulatory genes: (1) proto-oncogenes, which are cellular genes that regulate growth and differentiation; (2) tumor suppressor genes that inhibit growth, typically by regulating the cell cycle; (3) genes that regulate apoptosis (programmed cell death); (4) genes that mediate DNA repair. Carcinogenesis involves a multistep progression resulting from the cumulative effects of multiple mutations.

Once developed, neoplasms are: (1) self-sufficient, in that they have the capacity to proliferate without external stimuli; for example, as the result of unregulated oncogene activation; (2) insensitive to normal regulatory signals that would limit their growth, such as transforming growth factor and the cyclin-dependent kinases that normally regulate orderly progression of cells through the various phases of the cell cycle; (3) resistant to apoptosis because of either the activation of antiapoptotic molecules or the inhibition of mediators of apoptosis such as p53; (4) limitless potential for replication. Cancers also may have the ability to invade and spread to distant tissues (metastasis), and neoplasms typically promote the proliferation of new blood vessels that support their growth.

Neoplasia, regardless of cause, is the result of unregulated cellular proliferation. In the normal sequence of events during cellular proliferation, a growth factor binds to its specific cellular receptor, leading to signal transduction that ultimately results in nuclear transcription, which in turn leads to the cell entering and progressing through the cell cycle until it divides. Proto-oncogenes are normal cellular genes that encode proteins that function in normal cellular growth and differentiation; they include (1) growth factors; (2) growth factor receptors; (3) intracellular signal transducers; (4) nuclear transcription factors; (5) cell cycle control proteins. Oncogenes are derived by mutation of their normal cellular proto-oncogene counterparts, and the expression of oncogenes results in production of oncoproteins that mediate autonomous (unregulated) growth of neoplastic cells.

The development of cancer (malignant neoplasia) is a protracted, multistep process that reflects the accumulation of multiple mutations. Potentially neoplastic cells must bypass apoptosis (programmed death), circumvent the need for growth signals from other cells, escape from immunologic surveillance, organize their own blood supply, and possibly metastasize. Thus, tumors other than those induced by rapidly transforming retroviruses like Rous sarcoma virus generally do not arise as the result of a single event, but by a series of steps leading to progressively greater loss of regulation of cell division.

Viruses are classified as tumor viruses if part of the viral genome is present in tumors, with expression within the tumor of some viral genes. In vitro, infection of cells with tumor viruses leads to transformation caused by specific viral genes. Infection of experimental animals leads to tumor formation that is preventable by vaccination, although this experiment cannot be performed with most human viruses because they do not infect rodents. Oncogenic DNA viruses (eg, papillomaviruses, polyomaviruses, herpesviruses) and RNA viruses (retroviruses) have been identified in both animals and humans. DNA viruses can cause neoplasia by inhibiting tumor suppressor genes whereas RNA viruses typically activate proto-oncogenes. Cells transformed by nondefective retroviruses also express the full range of viral proteins, and new viruses bud from their membranes. In contrast, transformation by DNA viruses usually occurs in cells undergoing nonproductive infection in which viral DNA is integrated into the cellular DNA of the transformed cells or, in the case of papillomaviruses, polyomaviruses and herpesviruses, in which the viral DNA remains episomal. Certain virus-specific antigens are demonstrable in transformed cells.

Oncogenic RNA Viruses

Retrovirus-Induced Neoplasia

Retroviruses are a significant cause of neoplasia in many species of animals, including cattle, cats, nonhuman
viruses. The retroviral oncogene, \( v \)-onc, are directly oncogenic by carrying an additional viral gene that is \( v \)-onc. Acute transforming retroviruses infect mice and birds and originate from a host c-\( \text{onc} \) gene, where the transforming activity of the v-\( \text{onc} \) is accentuated by mutation. These mutations reflect the high error rate of the viral reverse transcriptase. Other viral oncogenes may induce cellular transformation simply by overexpression (from the viral promoter), independent of any mutations. These acquired genes are components of the cell signaling networks and the strongly promoted production of the viral oncprotein will readily exceed that of the normal cellular oncprotein. The result can be uncontrolled cell growth. Because c-\( \text{onc} \) genes are the precursors of v-\( \text{onc} \) genes, c-\( \text{onc} \) genes are also called “proto-oncogenes.” Wherever acute transforming retroviruses integrate in the host genome, it is the v-\( \text{onc} \) that is directly responsible for the rapid malignant change that occurs in cells infected with these viruses. Over 60 different v-\( \text{onc} \) genes have been identified, and retroviruses have been instrumental in identifying their cellular homologues.

The v-\( \text{onc} \) is usually incorporated into the viral genomic RNA, replacing a portion of one or more normal viral genes. Because such viruses have lost some of their viral genetic sequences, they are usually incapable of replication, and are therefore termed “defective” retroviruses. An exception is Rous sarcoma virus, in that its genome contains a viral oncogene (v-src) in addition to its full complement of functioning viral genes (\( \text{gag}, \text{pol}, \) and \( \text{env} \)); thus Rous sarcoma virus is both replication-competent and an acute transforming virus. Rous sarcoma virus is one of the most rapidly acting carcinogens known, transforming cultured cells in a day or so and causing neoplasia and death in chickens in as little as 2 weeks after infection. Defective retroviruses circumvent their defective replicative ability by utilizing nondefective “helper” retroviruses for formation of infectious virions. Replication of the defective virus is thus said to be “rescued” by helper viruses that provide the missing function (e.g., an environmentally stable envelope).

Although v-\( \text{onc} \) genes often compromise retrovirus replication, v-\( \text{onc} \) genes may be acquired over time by integrated proviruses, most likely because of the effects on cell proliferation that would amplify v-\( \text{onc} \) containing cells. Cell proliferation also favors replication of helper virus that can rescue the v-\( \text{onc} \) containing defective virus, thereby facilitating direct viral dissemination of v-\( \text{onc} \) within a host.

The various v-\( \text{onc} \) genes and the proteins they encode can be assigned to major classes: growth factors (such as v-sis); growth factor receptors and hormone receptors (such as v-\( \text{erbB} \)); intracellular signal transducers (such as v-ras); and nuclear transcription factors (such as v-jun). The oncprotein products of the various retroviral v-\( \text{onc} \) genes act in many different ways to affect cell growth, division, differentiation, and homeostasis:

1. v-\( \text{onc} \) genes usually contain only that part of their corresponding c-\( \text{onc} \) gene that is transcribed into messenger RNA—in most instances they lack the introns that are so characteristic of eukaryotic genes.
2. v-\( \text{onc} \) genes are separated from the cellular context that normally controls gene expression, including the normal promoters and other sequences that regulate c-\( \text{onc} \) gene expression.
3. v-\( \text{onc} \) genes are under the control of the viral long terminal repeats (LTRs), which not only are strong promoters but also are influenced by cellular regulatory factors. For some retrovirus v-\( \text{onc} \) genes, such as myc and mos, the presence of viral LTRs is all that is needed for tumor induction.
4. v-\( \text{onc} \) genes may undergo mutations (deletions and rearrangements) that alter the structure of their protein products; such changes can interfere with normal protein—protein interactions, leading to escape from normal regulation.
5. v-\( \text{onc} \) genes may be joined to other viral genes in such a way that their functions are modified. For example, in Abelson murine leukemia virus the v-\( \text{abl} \) gene is expressed as a fusion protein with a gag protein; this arrangement directs the fusion protein to the plasma membrane where the Abl protein functions. In feline leukemia virus, the v-\( \text{onc} \) gene fms is also expressed as a fusion protein with a gag protein, thus allowing the insertion of the Fms oncprotein in the plasma membrane.

Infection with acute transforming retroviruses may lead to transformation of every infected cell and therefore to very rapid tumor development (sometimes within days).
Chronic Transforming Retroviruses

Chronic transforming retroviruses induce neoplasia through integration into the genome of somatic cells. Recent research suggests that the selectivity of integration sites is specific for individual retrovirus species, and thereby contribute to pathogenicity. Chronic transforming retroviruses are classified as cis- or trans-acting. “Cis-acting” retroviruses (eg, avian leukemia viruses) transform cells by becoming integrated in the host-cell DNA close to a cell growth regulating gene, and thus usurping normal cellular regulation of this gene. These cell growth regulating host genes are termed “proto-oncogenes,” or cellular oncogenes (c-onc). Despite the terminology implying that they are oncogenic, c-onc genes are host genes that encode important cell signaling products that regulate normal cell proliferation and quiescence. The presence of an integrated provirus, with its strong promoter and enhancer elements, upstream from a c-onc gene may amplify the expression of the c-onc gene greatly. This is the likely mechanism whereby the weakly oncogenic endogenous avian leukemia viruses produce neoplasia. When avian leukemia viruses cause malignant neoplasia, the viral genome has generally been integrated at a particular location, immediately upstream from a host c-onc gene. Integrated avian leukemia provirus increases the synthesis of the normal c-myc oncogene product 30- to 100-fold. Experimentally, only the viral LTRs need be integrated to cause this effect; furthermore, by this mechanism c-myc may also be expressed in cells in which it is not normally expressed or is normally expressed at much lower levels. Infection with cis-acting retroviruses results in transformation of single cells (monoclonal tumor) and slow tumor formation over months.

“Trans-activating” retroviruses express viral proteins that act as oncogenes. The retroviruses that cause nasal carcinomas and pulmonary adenocarcinomas (Jaagsiekte) in sheep infect epithelial cells, and transformation is related to expression of the viral env gene. Bovine leukemia virus is an exogenous retrovirus that causes chronic leukemia and B cell lymphoma. Its Tax protein functions as a transactivator of host genes. Both the ovine retrovirus Env and the Tax proteins of bovine leukemia virus stimulate continuous cell division of infected cells, which is thought to result in an increased number of mutations and subsequently cellular transformation. Infection with trans-acting retroviruses leads to oligoclonal tumors which develop over months to years.

Oncogenic DNA Viruses

Apart from retroviruses, the most important oncogenic viruses in animals are DNA viruses (papillomaviruses, polyomaviruses, herpesviruses; see also Table 3.4). DNA tumor viruses interact with cells in one of two ways: (1) productive infection, in which the virus completes its replication cycle, resulting in cell lysis or (2) nonproductive infection, in which the virus transforms the cell without completing its replication cycle. During such nonproductive infection, the viral genome or a truncated version of it is integrated into the cellular DNA or the complete genome persists as an autonomously replicating episome (episome). The genome continues to express early genes. The molecular basis of oncogenesis by DNA viruses is best understood for polyomaviruses, papillomaviruses, and adenoviruses, all of which contain genes that behave as oncogenes, including tumor suppressor genes. These oncogenes appear to act by mechanisms similar to those described for retrovirus oncogenes: they act primarily in the nucleus, where they alter patterns of gene expression and regulation of cell growth. The relevant proteins have a dual role in both virus replication and cell transformation. With a few possible exceptions, the oncogenes of DNA viruses have no homologue or direct ancestors (c-onc genes) among cellular genes of the host.

Oncogenic Papillomaviruses

Papillomaviruses produce papillomas (warts) on the skin and mucous membranes of most animal species (see Chapter 11: Papillomaviridae and Polyomaviridae). Papillomas are hyperplastic epithelial outgrowths that generally regress spontaneously. Occasionally, however, infections by some papillomavirus types may cause malignant cellular transformation, resulting in the development of cancer. Papillomaviruses are known to cause oropharyngeal and cervical squamous cell carcinomas in people. In animals, papillomaviruses are also thought to cause sarcomas in horses, and have been associated with some squamous cell carcinomas in horses, cats and dogs.

In warts, the papillomavirus DNA remains episomal, meaning it is not integrated into the host-cell DNA and persists as an autonomously replicating episome. In contrast, in human papillomavirus-induced neoplasms the viral DNA is integrated into that of the host. As the pattern of integration is clonal within cancers, each cancer cell carries at least one, and often many incomplete copies of the viral genome. The site of virus integration is random, and there is no consistent association with cellular proto-oncogenes. For some papillomaviruses, integration disrupts one of the early genes, E2, which is a viral repressor. Other viral genes may also be deleted, but the viral oncogenes (eg, E6 and E7) remain intact. These oncogenes alter normal cell growth and division and the overexpression of E6 and E7 is considered a critical step in malignant transformation by a human papillomavirus. It is to be stressed that the development of warts is a normal part of viral replication cycle of some papillomavirus
### TABLE 3.4 Viruses That Can Induce Tumors in Domestic or Laboratory Animals

| DNA Viruses |  |  |
|-------------|-----------------|-----------------|
| **Poxviridae/Leporipoxvirus** | Rabbit fibroma virus and squirrel fibroma virus | Fibromas and myxomas in rabbits and squirrels (hyperplasia rather than neoplasia) |
| **Poxviridae/Yatapoxvirus** | Yaba monkey tumor virus | Histiocytoma in monkeys |
| **Herpesviridae/Alphaherpesvirinae/Mardivirus** | Marek’s disease virus | T cell lymphoma in fowl |
| **Herpesviridae/Herpesviridae/Cryptomavirinae** | Epstein–Barr virus | Burkitt’s lymphoma, nasopharyngeal carcinoma, and B cell lymphomas in humans and monkeys |
| Baboon herpesvirus | Lymphoma in baboons |
| **Herpesviridae/Herpesviridae/Gammaherpesvirinae/Lymphocryptovirus** | Cottontail rabbit herpesvirus | Lymphoma in rabbits |
| **Alloherpesviridae/ranid herpesvirus** | Lucké frog herpesvirus | Renal adenocarcinoma in frogs and tadpoles |
| **Papillomaviridae/multiple genera** | Cottontail rabbit papillomavirus | Papillomas, skin cancers in rabbits |
| Bovine papillomavirus 4 | Papillomas, carcinoma of intestine, bladder |
| Bovine papillomavirus 7 | Papillomas, carcinoma of eye |
| Equine papillomavirus | Squamous cell carcinoma |
| **Polyomaviridae/Polyomavirus** | Raccoons | Central nervous system |
| Murine polyomavirus | Tumors in newborn rodents |

### Reverse Transcribing Viruses

| Hepadnaviridae/Orthohepadnavirus | Human, woodchuck hepatitis viruses | Hepatocellular carcinomas in humans and woodchucks |
|----------------------------------|----------------------------------|-----------------------------------------------|
| **Hepadnaviridae/Avihepadnavirus** | Duck hepatitis virus | Hepatocellular carcinomas in ducks |
| **Retroviridae/Alpharetrovirus** | Avian leukosis viruses | Leukosis (lymphoma, leukemia), osteopetrosis, nephroblastoma in fowl |
| Rous sarcoma virus | Sarcoma in fowl |
| Avian myeloblastosis virus | Myeloblastosis in fowl |
| **Retroviridae/Betaretrovirus** | Mouse mammary tumor virus | Mammary carcinoma in mice |
| Mason–Pfizer monkey virus | Sarcoma and immunodeficiency disease in monkeys |
| Ovine pulmonary adenocarcinoma virus (jaagsiekte virus) | Pulmonary adenocarcinoma in sheep |
| **Retroviridae/Gammaretrovirus** | Feline leukemia virus | Leukemia in cats |
| Feline sarcoma virus | Sarcoma in cats |
| Murine leukemia and sarcoma viruses | Leukemia, lymphoma, and sarcoma in mice |
| **Retroviridae/Deltaretrovirus** | Avian reticuloendotheliosis virus | Reticuloendotheliosis in fowl |
| Bovine leukemia virus | Leukemia (B cell lymphoma) in cattle |

*Not true oncogenic viruses. They differ from all other viruses listed in that poxviruses replicate in cytoplasm and do not affect the cellular genome.*
types. However, the integration of DNA into a cell is accidental and prevents replication of the papillomavirus and only a very small proportion of papillomavirus infections result in cancer development. However, bovine papillomavirus type 1 is thought to cause equine sarcomas predominantly through changes in cell proliferation that are mediated by the E5 oncoprotein. In contrast to human papillomavirus-induced cancers, viral integration appears to be uncommon within papillomavirus-associated cancers in animals.

**Oncogenic Hepadnaviruses**

Mammalian, but not avian, hepadnaviruses are associated strongly with naturally occurring hepatocellular carcinomas in their natural hosts. Woodchucks that are chronically infected with woodchuck hepatitis virus almost inevitably develop hepatocellular carcinoma, even in the absence of other carcinogenic factors. Oncogenesis induced by mammalian hepadnaviruses is a multifactorial process, and there are differences in the cellular mechanisms responsible for carcinogenesis associated with different viruses. Whereas ground squirrel and woodchuck hepatitis viruses activate cellular oncogenes, the mode of action of human hepatitis B virus is uncertain, as it apparently has no consistent site of integration or oncogene association. The hepatocellular regeneration accompanying cirrhosis of the liver also promotes the development of neoplasia in hepatitis virus-infected humans, but there is no cirrhosis in the animal models. The likelihood of hepadnavirus-associated carcinoma is greatest in animals (and humans) infected at birth.

**Oncogenic Poxviruses**

Although some poxviruses are regularly associated with the development of benign tumor-like lesions (see Chapter 7: Poxviridae), there is no evidence that these ever become malignant, nor is there evidence that poxvirus DNA is ever integrated into cellular DNA. A very early viral protein produced in poxvirus-infected cells displays homology with epidermal growth factor and is probably responsible for the epithelial hyperplasia characteristic of many poxvirus infections. For some poxviruses (eg, fowlpox, orf, and rabbit fibroma viruses), epithelial hyperplasia is a dominant clinical manifestation and may be a consequence of a more potent form of the poxvirus epidermal growth factor homologue.

**Oncogenic in Experimental Systems: Polyomaviruses and Adenoviruses**

During the 1960s and 1970s, two members of the family Polyomaviridae, murine polyomavirus and simian virus 40 (SV40), as well as certain human adenoviruses (types 12, 18, and 31) were shown to induce malignant neoplasms following their inoculation into baby hamsters and other rodents. With the exception of murine polyomavirus, none of these viruses induces cancer under natural conditions in its natural host, rather they transform cultured cells of certain other species and provide experimental models for analysis of the molecular events in cell transformation. More recently, polyomaviruses have been incriminated as the cause of cancers in both humans and animals (see Chapter 11: Papillomaviridae and Polyomaviridae).

Polyomavirus- or adenovirus-transformed cells do not produce virus. Viral DNA is integrated at several sites in the chromosomes of the cell. Most of the integrated viral genomes are complete in the case of the polyomaviruses, but defective in the case of the adenoviruses. Only certain early viral genes are transcribed, albeit at an unusually high rate. By analogy with retrovirus genes, they are now called oncogenes. Their products, demonstrable by immuno-fluorescence, used to be known as tumor (T) antigens. A great deal is now known about the role of these proteins in transformation. Virus can be rescued from polyomavirus-transformed cells—that is, virus can be induced to replicate by irradiation, treatment with certain mutagenic chemicals, or cocultivation with certain types of permissive cells. This cannot be done with adenovirus-transformed cells, as the integrated adenovirus DNA contains substantial deletions.