CHAPTER 2

An Outline of Virus Replication and Viral Pathogenesis

Viruses must replicate in living cells. The most basic molecular requirement for virus replication is for viruses to induce either profound or subtle changes in the cell so that viral genes in the genome are replicated and viral proteins are expressed. This will result in the formation of new viruses—usually many more than the number of viruses infecting the cell in the first place. When replicating, viruses use portions of the cell's equipment for replication of viral nucleic acids and expression of viral genes, all of the cell's protein synthetic machinery, and all of the cell's energy stores that are generated by its own metabolic processes.

The dimensions and organization of "typical" animal, plant, and bacterial cells are shown in Fig. 2.1. The size of a typical virus falls in the range between the diameters of a ribosome and of a centriolar filament. With most viruses, infection of a cell with a single virus particle will result in the synthesis of more (often by a factor of several powers of 10) infectious virus. Any infection that results in the production of more infectious virus at the end than at the start is classified as a **productive infection**. The actual number of infectious viruses produced in an infected cell is called the **burst size**, and this number can range from less than 10 to over 10,000, depending on the type of cell infected, nature of the virus, and many other factors.

Infections with many viruses completely convert the cell into a factory for replication of new viruses. Infection with some types of viruses, however, can lead to a situation where the cell and virus coexist for periods of time, which can be as long as the life of the host. This process can be a dynamic one in which there is a small amount of virus produced constantly, or it can be passive where the viral genome is carried as a "passenger" in the cell with little or no evidence of viral gene expression. Often in such a case the virus induces some type of change in the cell so that the viral and cellular genomes are replicated in synchrony. Such coexistence usually results in accompanying changes to protein composition of the cell's surface—the immune "signature" of the cell—and often there are functional changes as well. This process is called **lysogeny** in bacterial cells and **transformation** in animal and plant cells.

In animal cells, the process of transformation often results in altered growth properties of the cell and can result in the generation of cells that have some or many properties of **cancer cells**. There are instances, however, where the coexistence of a cell and an infecting virus leads to few or no

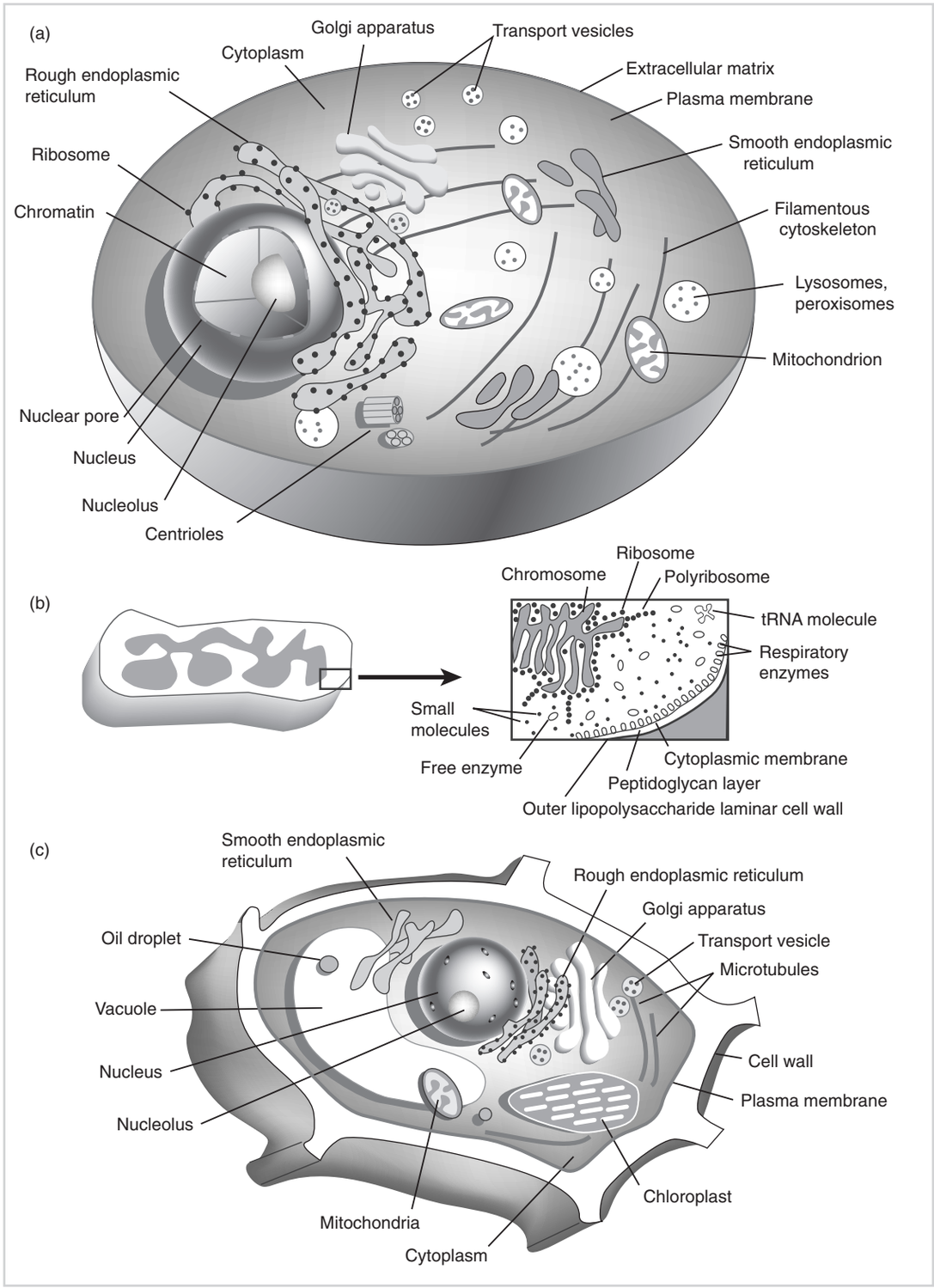

Fig. 2.1 Dimensions and features of "typical" animal (*a*), bacterial (*b*), and plant (*c*) cells. The dimensions of plant and animal cells can vary widely, but an average diameter of around 50 µm (5×10^{-5} m) is a fair estimate. Bacterial cells also show great variation in size and shape, but the one shown here is *Escherichia coli*, the true "workhorse" of molecular biologists. Its length is approximately 5 µm. Based on these dimensions and shapes of the cells shown, the bacterial cell is on the order of 1/500th of the volume of the eukaryotic cell shown. Virus particles also vary greatly in size and shape, but generally range from 25 to 200 nm ($0.25–2.00 \times 10^{-7}$ m).

detectable changes in the cell. For example, **herpes simplex virus** (**HSV**) can establish a **latent infection** in terminally differentiated sensory neurons. In such cells there is absolutely no evidence for expression of any viral protein at all. Periods of viral latency are interspersed with periods of **reactivation** (**recrudescence**) where virus replication is reestablished from the latently infected tissue for varying periods of time.

Some viral infections of plant cells also result in stable association between virus and cell. Indeed, the variegation of tulip colors, which led to economic booms in Holland during the sixteenth century, is the result of such associations. Many other examples of **mosaicism** resulting from persisting virus infections of floral or leaf tissue have been observed in plants. However, many specific details of the association are not as well characterized in plants as in animal and bacterial cells.

Virus replication in the cell

Various patterns of replication as applied to specific viruses, as well as the effect of viral infections on the host cell and organism, are the subject of many of the following chapters in this book. The best way to begin to understand patterns of virus replication is to consider a simple general case: the **productive infection** cycle. A number of critical events are involved in this cycle. The basic pattern of replication is as follows:

1 The virus specifically interacts with the host cell surface, and the viral genome is introduced into the cell. This involves specific recognition between virus surface proteins and specific proteins on the cell surface (**receptors**) in animal and bacterial virus infections.

2 Viral genes are expressed using host cell processes. This viral gene expression results in synthesis of a few or many viral proteins involved in the replication process.

3 Viral proteins modify the host cell and allow the viral genome to replicate using host and viral enzymes. While this is a simple statement, the actual mechanisms by which viral enzymes and proteins can subvert a cell are manifold and complex. This is often the stage at which the cell is irreversibly modified and eventually killed. Much modern research in the molecular biology of virus replication is directed toward understanding these mechanisms.

4 New viral coat proteins assemble into capsids and viral genomes are included. The process of assembly of new virions is relatively well understood for many viruses. The successful description of the process has resulted in a profound linkage of knowledge about the principles of macromolecular structures, the biochemistry of protein–protein and protein–nucleic acid interactions, and an understanding of the thermodynamics of large macromolecule structure.

5 Virus is released where it can infect new cells and repeat the process. This is the basis of virus spread, whether from cell to cell or from individual to individual. Understanding the process of virus release requires knowledge of the biochemical interactions between cellular organelles and viral structures. Understanding the process of virus spread between members of a population requires knowledge of the principles of epidemiology and public health.

PATHOGENESIS OF VIRAL INFECTION

Most cells and organisms do not passively submit to virus infection. As noted in the previous chapter, the response of organisms to virus infection is a major feature of evolutionary change in its most general sense. As briefly noted, a complete understanding of pathogenesis requires knowledge of the sum total of genetic features a virus encodes that allows its efficient spread between individual hosts and within the general population of hosts. Thus, the term *pathogenesis* can be legitimately applied to virus infections of multicellular, unicellular, and bacterial hosts.

A major challenge for viruses infecting bacteria and other unicellular organisms is finding enough cells to replicate in without isolating themselves from other populations of similar cells. In

other words, they must be able to "follow" the cells to places where the cells can flourish. If susceptible cells can isolate themselves from a pathogen, it is in their best interest to do so. Conversely, the virus, even constrained to confine all its dynamic features of existence to the replication process per se, must successfully counter this challenge or it cannot survive.

In some cases, cells can mount a defense against virus infection. Most animal cells react to infection with many viruses by inducing a family of cellular proteins termed *interferons* that can interact with neighboring cells and induce those cells to become wholly or partially resistant to virus infection. Similarly, some viral infections of bacterial cells can result in a **bacterial restriction** response that limits viral replication. Of course, if the response is completely effective, the virus cannot replicate. In this situation, one cannot study the infection, and in the extreme situation, the virus would not survive.

Viruses that infect multicellular organisms face problems attendant with their need to be introduced into an animal to generate a physiological response fostering the virus's ability to spread to another organism (i.e., they must exhibit virulence). This process can follow different routes.

Disease is a common result of the infection, but not all viral infections result in measurable disease symptoms. Inapparent or **asymptomatic** infections can result from many factors. Some infections are influenced by the host's genetic makeup, some are reflective of viral gene function, and some are due to the random (**stochastic**) nature of the infective process.

Stages of infection

Pathogenesis can be divided into stages—from initial infection of the host to its eventual full or partial recovery, or its virus-induced death. A more-or-less prototypical course of infection in a vertebrate host is schematically diagrammed in Fig. 2.2. Although individual cases differ, depend-

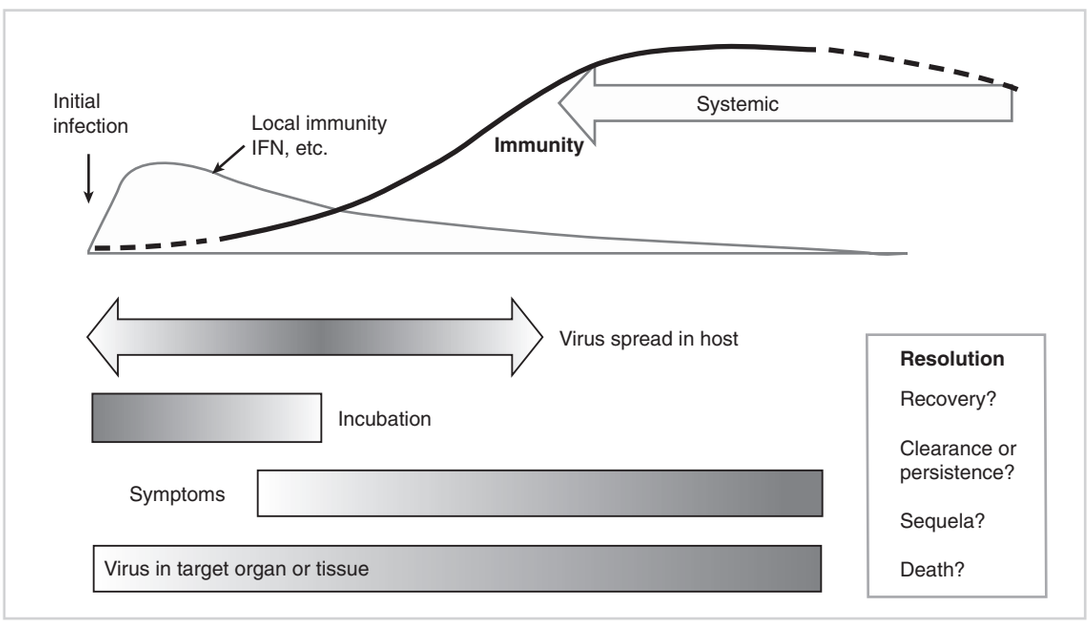

Fig. 2.2 The pathogenesis of virus infection. Typically, infection is followed by an incubation period of variable length in which virus multiplies at the site of initial infection. Local and innate immunity including the interferon response counter infection from the earliest stages, and if these lead to clearing, disease never develops. During the incubation period, virus spreads to the target of infection (which may be the same site). The adaptive immune response becomes significant only after virus reaches high enough levels to efficiently interact with cells of the immune system; this usually requires virus attaining high levels or titers in the circulatory system. Virus replication in the target leads to symptoms of the disease in question, and is often important in spread of the virus to others. Immunity reaches a maximum level only late in the infection process, and remains high for a long period after resolution of the disease.

ing on the nature of the viral pathogen and the immune capacity of the host, a general pattern of infection would be as follows.

Initial infection leads to virus replication at the site of entry and multiplication and spread into favored tissues. The time between the initial infection and the observation of clinical symptoms of disease defines the **incubation period**, which can be of variable length, depending on many factors.

The host responds to the viral invasion by marshaling its defense forces, both local and systemic. The earliest defenses include expression of interferon, and ultimately, the major component of this defense, **adaptive immunity**. For disease to occur, the defenses must lag as the virus multiplies to high levels. At the same time, the virus invades favored sites of replication. Infection of these favored sites is often a major factor in the occurrence of disease symptoms and is often critical for the transmission to other organisms. As the host defenses mount, virus replication declines and there is recovery—perhaps with lasting damage and usually with immunity to a repeat infection. If an insufficient defense is mounted, the host will die.

Initial stages of infection—entry of the virus into the host

The source of the infectious virus is termed the **reservoir**, and virus entry into the host generally follows a specific pattern leading to its introduction at a specific site or region of the body. Epidemiologists working with human, animal, and plant diseases often use special terms to describe parts of this process. The actual means of infection between individuals is termed the *vector of transmission* or, more simply, the **vector**. This term is often used when referring to another organism, such as an arthropod, that serves as an intermediary in the spread of disease.

Many viruses must continually replicate to maintain themselves—this is especially true for viruses that are sensitive to desiccation and are spread between terrestrial organisms. For this reason, many virus reservoirs will be essentially dynamic; that is, the virus constantly must be replicating actively somewhere. In an infection with a virus with broad species specificity, the external reservoir could be a different population of animals. In some cases, the vector and the reservoir are the same—for example, in the transmission of rabies via the bite of a rabid animal. Also, some arthropod-borne viruses can replicate in the arthropod vector as well as in their primary vertebrate reservoir. In such a case the vector serves as a secondary reservoir, and this second round of virus multiplication increases the amount of pathogen available for spread into the next host.

Some reservoirs are not entirely dynamic. For example, some algal viruses exist in high levels in many bodies of freshwater. It has been reported that levels of some viruses can approach 10^7 per milliliter of seawater. Further, the only evidence for the presence of living organisms in some bodies of water in Antarctica is the presence of viruses in that water. Still, ultimately, all viruses must be produced by an active infection somewhere, so in the end all reservoirs are, in some sense at least, dynamic.

Viruses must replicate in cells via interaction with receptors on a hydrated cell surface. Thus, initial virus infection and entry into the host cell must take place at locations where such cell surfaces are available, not, for example, at the desiccated surface layer of keratinized, dead epithelial cells of an animal, or at the dry, horny surface of a plant. In other words, virus must enter the organism at a site that is "wet" as a consequence of its anatomical function or must enter through a trauma-induced break in the surface. Figure 2.3 is a schematic representation of some modes of virus entry leading to human infection.

The incubation period and spread of virus through the host

Following infection, virus must be able to replicate at the site of initial infection in order for it to build up enough numbers to lead to the symptoms of disease. There are several reasons why this

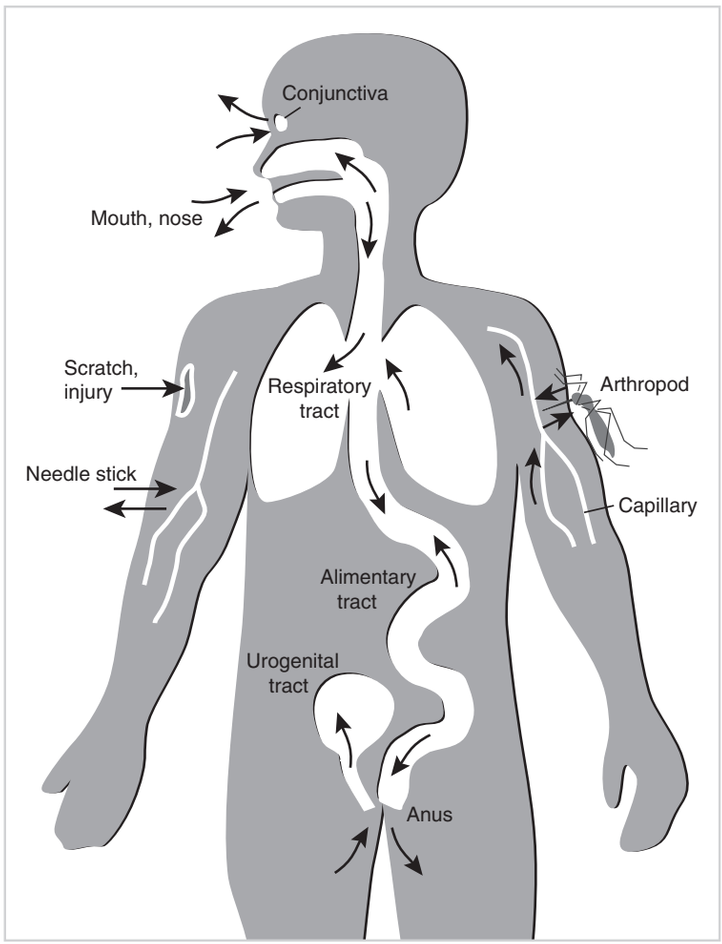

Fig. 2.3 Sites of virus entry in a human. These or similar sites apply to other vertebrates. (Adapted from Mims, C. A., and White, D. O. *Viral Pathogenesis and Immunology*. Boston: Blackwell Science, 1984.)

takes time. First, only a limited amount of virus can be introduced. This is true even with the most efficient vector. Second, cell-based innate immune responses occur immediately upon infection. The best example of these is the interferon response.

This "early" stage or incubation period of disease can last from only a few days to many years, depending on the specific virus. In fact, probably many virus infections go no further than this first stage, with clearance occurring without any awareness of the infection at all. Also, some virus infections lead only to replication localized at the site of original entry. In such a case, extensive virus spread need not occur, although some interaction with cells of the immune system must occur if the animal host is to mount an immune response.

Following entry, many types of viruses must move or be moved through the host to establish infection at a preferred site, the infection of which results in disease symptoms. This site, often referred to as the **target tissue** or **target organ**, is often (but not always) important in mediating the symptoms of disease, or the spread, or both.

There are several modes of virus spread in the host. Perhaps the most frequent mode utilized by viruses is through the circulatory system (**viremia**). A number of viruses can spread in the bloodstream either passively as free virus or adsorbed to the surface of cells that they do not infect, such as red blood cells. Direct entry of virus into the lymphatic circulatory system also can lead to viremia. Some viruses that replicate in the gut (such as poliovirus) can directly enter the lymphatic system

Table 2.1 Some viruses that replicate in cells of the lymphatic system.

Cells Infected	Virus
B lymphocytes	Epstein-Barr virus (herpesvirus)
	Some retroviruses
T lymphocytes	Human T-cell leukemia virus
	HIV
	Human herpesvirus 6
	Human herpesvirus 7
Monocytes	Measles virus
	Varicella-zoster virus (herpesvirus)
	HIV
	Parainfluenza virus
	Influenza virus
	Rubella (German measles) virus
	Cytomegalovirus (herpesvirus)

via **Peyer's patches** (**gut-associated lymphatic tissue**) in the intestinal mucosa. Such patches of lymphatic tissue provide a route directly to lymph nodes without passage through the bloodstream. This provides a mode of generating an immune response to a localized infection. For example, poliovirus generally replicates in the intestinal mucosa and remains localized there until eliminated; the entry of virus into the lymphatic system via Peyer's patches leads to immunity. Virus invasion of gut-associated lymphatic tissue is thought to be one important route of entry for HIV spread by anal intercourse, as infectious virus can be isolated from seminal fluid of infected males.

Infection of lymphatic cells can also be a factor in the spread of infectious virus. HIV infects and replicates in macrophages, leading to the generation of active carrier cells that migrate to lymph nodes. This facilitates spread of the virus to the immune system. Many other viruses infect and replicate in one or another cells of the lymphatic system. Some of the viruses known to infect one or another of the three major cells found in lymphatic circulation are shown in Table 2.1.

While spread via the circulatory system is quite common, it is not the only mode of general dissemination of viruses from their site of entry and initial replication in animals. The nervous system provides the other major route of spread. Some **neurotropic viruses**, such as HSV and rabies virus, can spread from the peripheral nervous system directly into the central nervous system (**CNS**). In the case of HSV, this is a common result of infection in laboratory mice; however, it is a relatively rare occurrence in humans, and is often correlated with an impairment or lack of normal development of the host's immune system. Thus, an initial acute infection of an infant at the time of birth or soon thereafter can lead to HSV encephalitis with high frequency.

Multiplication of virus to high levels—occurrence of disease symptoms

Viral replication at specific target tissues often defines **symptoms** of the disease. The nature of the target and the host response are of primary importance in establishing symptoms. The ability of a virus to replicate in a specific target tissue results from specific interactions between viral and cellular proteins. In other words, one or another viral protein can recognize specific molecular features that define those cells or tissues favored for virus replication. These virus-encoded proteins, thus, have a major role in specifying the virus's tissue **tropism**. Host factors, such as speed of immune response and inflammation, also play a major role. For example, a head cold results from infection and inflammation of the nasopharynx. Alternatively, liver malfunction due to inflammatory disease (*hepatitis*) could result from an infection in this critical organ.

One major factor in viral tropism is the distribution and occurrence of specific viral receptors on cells in the target tissue. The role of such receptors in the infection process is described in Chapter 6. For the purposes of the present discussion, it is enough to understand that there must be a specific and spatially close interaction between proteins at the surface of the virus and the surface of the cell's plasma membrane for the virus to be able to begin the infection process.

One example of the role of receptors in tissue tropism involves the poliovirus receptor, which is found on cells of the intestinal mucosa and in lymphatic tissue. A related molecule is also present on the surface of motor neurons, which means that neurotropic strains of poliovirus can invade, replicate in, and destroy these cells under certain conditions of infection. In another example, HIV readily infects **T lymphocytes** by recognizing the $CD4^+$ surface protein in association with another specific **chemokine** receptor that serves as a coreceptor. Rabies virus's ability to remain associated with nervous tissue probably is related to its use of the acetylcholine receptor present at nerve cell synapses. The ability of *vaccinia* virus (like the related smallpox virus) to replicate in epidermal cells is the result of its use of the epidermal growth factor receptor on such cells as its own receptor for attachment.

While tissue tropism is often understandable in terms of a specific viral receptor being present on the surface of susceptible cells, the story can be quite complicated in practice. The natural course of HIV infection involves infection of neural cells, but no protein is clearly established as related to the HIV receptor on neural cells. It may be that the close interaction between HIV-infected **microglial cells** (cells of the CNS with some functions and properties similar to T lymphocytes) can lead to alternative modes of viral entry. Alternatively, it has been recently shown that HIV will bind to galactosyl ceramide (GalC), a structure found on the surface of many cells in the CNS. Microglial cell infection is mediated by the co-receptor, CCR5.

This may also be the case for infections with Epstein-Barr virus (**EBV**), which is found in **B lymphocytes** in patients who have been infected with the virus. It is thought that primary infection of epithelial cells in the mucosa of the nasopharynx, followed by association with lymphocytes during development of the immune response, leads to infection of B cells that carry the EBV-specific CD21 receptor.

Even though the infection of target tissue is usually associated with the occurrence of virus infection symptoms, the target is not always connected with the spread of a virus infection. For example, HIV infection can be readily spread from an infected individual long before any clinical symptoms of the disease (AIDS) are apparent. An individual who has undergone a subclinical reactivation episode where there is virus in the saliva, but no fever blister can transmit HSV. Finally, paralytic polio is the result of a "dead-end" infection of motor neurons, and the resulting death of those neurons and paralysis has nothing to do with spread of the virus.

The later stages of infection — the immune response

Infections with virus do not necessarily lead to any or all the symptoms of a disease. The severity of such symptoms is a function of the virus genotype, the amount of virus inoculum delivered to the host, and the host's general immune competence — the factors involved with virulence of the infection. The same virus in one individual can lead to an infection with such mild symptoms of disease that they are not recognized for what they are, while infection of another individual can lead to severe symptoms.

Generally, a virus infection results in an effective and lasting immune response. This is described in more detail in Chapter 7; briefly, the host's immune response (already activated by the presence of viral **antigens** at any and all sites where virus is replicating) reaches its highest level as clinical signs of the disease manifest.

A full immune response to virus infection requires the maturation of B and T lymphocytes. The maturation of lymphocytes results in the production of short-lived **effector T cells**, which kill cells

expressing foreign antigens on their surfaces. Another class of effector T cells helps in the maturation of effector B cells for the secretion of antiviral antibodies. Such a process takes several days to a week after stimulation with significant levels of viral antigen. An important part of this immune response is the generation of long-lived memory lymphocytes to protect against future reinfection.

In addition to the host's immune response, which takes some time to develop, a number of nonspecific host responses to infection aid in limitation of the infection and contribute to virus clearing. Interferon quickly renders sensitive cells resistant to virus infection; therefore, their action limits or interferes with the ability of the virus to generate high yields of infectious material. Other responses include tissue inflammation, macrophage destruction of infected cells, and increases in body temperature, which can result in suboptimal conditions for virus infection.

The later stages of infection—virus spread to the next individual

Virus exit is essentially the converse of virus entry at the start of the infection. Now, however, the infected individual is a reservoir of the continuing infection, and symptoms of the disease may have a role in its spread. Some examples should illustrate this simple concept. Infection with a mosquito-borne encephalitis virus results in high **titers** of virus in the victim's blood. At the same time, the infected individual's malaise and torpor make him or her an easy mark for a feeding mosquito. In **chicken pox** (caused by **herpes zoster virus** also called **varicella zoster virus** [**VZV**]), rupture of virus-filled **vesicles** at the surface of the skin can lead to generation of viral aerosols that transmit the infection to others. Similarly, a respiratory disease-causing virus in the respiratory tract along with congestion can lead to sneezing, an effective way to spread an aerosol. A virus such as HIV in body fluids can be transmitted to others via contaminated needles or through specific sexual practices such as unprotected intercourse, especially anal intercourse. Herpesvirus in saliva can enter a new host through a small crack at the junction between the lip and the **epidermis**.

The later stages of infection—fate of the host

Following a viral or any infectious disease, the host recovers or dies. While many **acute infections** result in clearance of virus, this does not invariably happen. While infections with influenza virus, cold viruses, polioviruses, and poxviruses resolve with virus clearance, herpesvirus infections result in a lifelong latent infection. During the latent period, no infectious virus is present, but viral genomes are maintained in certain protected cells. Periodically, a (usually) milder recurrence of the disease (reactivation or recrudescence) takes place upon suitable stimulation.

In distinct contrast, *measles* infection resolves with loss of infectious virus, but a portion of the viral genome can be maintained in neural tissue. This is not a latent infection because the harboring cells can express viral antigens, which lead to lifelong immunity, but infectious virus can never be recovered.

Other lasting types of virus-induced damage can be much more difficult to establish without extensive epidemiological records. Chronic liver damage due to hepatitis B virus infection is a major factor in hepatic carcinoma. Persistent virus infections can lead to immune dysfunction. Virus infections may also result in the appearance of a disease or **syndrome** (a set of diagnostic symptoms displayed by an affected individual) years later that has no obvious relation to the initial infection. It has been suggested that diseases such as diabetes mellitus, multiple sclerosis, and rheumatoid arthritis have viral **etiologies** (ultimate causative factors). Virus factors have also been implicated in instances of other diseases such as cancer and schizophrenia.

QUESTIONS FOR CHAPTER 2

1 A good general rule concerning the replication of RNA viruses is that they require what kind of molecular process?

2 What is the role of a vector in the transmission of a viral infection?

3 It is said that viruses appear to "violate the cell theory" ("cells only arise from preexisting cells"). To which phase of a virus life cycle (growth curve) does this refer? What is the explanation for this phase of the growth curve?

4 Viruses are called "obligate intracellular parasites." For which step of gene expression do *all* viruses completely depend on their host cell?

5 Viruses are said to "violate the cell theory," indicating that there are differences between viruses and cells. In the following table are listed several features of either viruses or cells or both. Indicate which of these features is true for viruses and which for cells. Write a "Yes" if the feature is *true* or a "No" if the feature is *not true* in each case.

Feature	Cells	Viruses
The genetic information may be RNA rather than DNA.		
New individuals arise by binary fission of the parent.		
Proteins are translated from messenger RNAs.		
New individuals assemble by spontaneous association of subunit structures.		