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CHAPTER 7

Pathogenesis

WHAT'S IN THIS CHAPTER?

- We start by considering the link between virus infection and disease.
- Next we describe how virus infection may injure the body, including how HIV infection causes AIDS and how some viruses may cause cancer.
- We finish by looking at emergent viruses and consider what the future may hold for us.

CONTENTS

Pathogenicity, the capacity of one organism to cause disease in another, is a complex and variable process. For one thing, it is rather difficult to define. At the simplest level there is the question of defining what disease is. An all-embracing definition would be that disease is a departure from the normal physiological parameters of an organism. This could range from a temporary and very minor condition, such as a slightly raised temperature or lack of energy, to chronic pathologic conditions that eventually result in death. Any of these conditions may result from a tremendous number of internal or external sources. There is rarely one single factor that causes a disease. Most disease states are multifactorial at some level.

In considering virus diseases, two aspects are involved, the direct effects of virus replication and the effects of body's responses to the infection. The course of any virus infection is determined by a delicate and dynamic balance between the host and the virus, as described in Chapter 6. The extent and severity of virus pathogenesis is determined similarly. In some virus infections, most of the pathologic symptoms observed are not directly caused by virus replication but are the side effects of the immune response. Inflammation, fever, headaches, and skin rashes are not usually caused by viruses themselves but by the cells of the immune system due to the release of potent chemicals such as interferons and interleukins. In the most extreme cases, it is possible that none of the pathologic effects of certain diseases is caused directly by the virus, except that its presence stimulates activation of the immune system.

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In the past few decades, molecular genetic analysis has contributed enormously to our understanding of virus pathogenesis. Nucleotide sequencing and site-directed mutagenesis have been used to explore molecular determinants of virulence in many different viruses. Specific sequences and structures found only in disease-causing strains of viruses and not in closely related attenuated or **avirulent** strains have been identified. Sequence analysis has also led to the identification of T-cell and B-cell epitopes on virus proteins responsible for their recognition by the immune system. Unfortunately, these advances do not automatically lead to an understanding of the mechanisms responsible for pathogenicity.

Unlike the rest of this book, this chapter is specifically about viruses that cause disease in animals. It does not discuss viruses that cause disease in plants, which have already been considered in Chapter 6. Three major aspects of virus pathogenesis are considered: direct cell damage resulting from virus replication, damage resulting from immune activation or suppression, and cell **transformation** caused by viruses.

BOX 7.1. DON'T BLAME THE VIRUSES!

Virus pathogenesis is an abnormal and fairly rare situation. The majority of virus infections are silent and do not result in any outward signs of disease. It is sometimes said that viruses would disappear if they killed their hosts. This is not necessarily true. It is possible to imagine viruses with a hit-and-run strategy, moving quickly from one dying host to the next and relying on continuing circulation for their survival. Nevertheless, there is a clear tendency for viruses not to injure their hosts if possible. A good example of this is the rabies virus. The symptoms of human rabies virus infections are truly dreadful, but thankfully rare. In its normal hosts (e.g., foxes), rabies virus infection produces a much milder disease that does not usually kill the animal. Humans are an unnatural, dead-end host for this virus, and the severity of human rabies is as extreme as the condition is rare.

Ideally, a virus would not even provoke an immune response from its host, or at least would be able to hide to avoid the effects. Herpesviruses and some retroviruses have evolved complex lifestyles that enable them to get close to this objective, remaining silent for much of the time. Of course, fatal infections such as rabies and acquired immune deficiency syndrome (AIDS) always grab the headlines. Much less effort has been devoted to isolating and studying the many viruses that have not (yet) caused well-defined diseases in humans, domestic animals, or economically valuable crop plants.

MECHANISMS OF CELLULAR INJURY

Virus infection often results in a number of changes that are detectable by visual or biochemical examination of infected cells. These changes result from the production of virus proteins and nucleic acids, but also from alterations to the biosynthetic capabilities of infected cells. Virus replication sequesters cellular

apparatus such as ribosomes and raw materials that would normally be devoted to synthesizing molecules required by the cell. **Eukaryotic** cells must carry out constant macromolecular synthesis, whether they are growing and dividing or in a state of quiescence. A growing cell clearly needs to manufacture more proteins, more nucleic acids, and more of all of its components to increase its size before dividing. However, there is a more fundamental requirement for such continuous activity. The function of all cells is regulated by controlled expression of their genetic information and the subsequent degradation of the molecules produced. Such control relies on a delicate and dynamic balance between synthesis and decay, which determines the intracellular levels of all the important molecules in the cell. This is particularly true of the control of the cell cycle, which determines the behavior of dividing cells (see "Cell Transformation by DNA Viruses," later). In general terms, a number of common phenotypic changes can be recognized in virus-infected cells. These changes are often referred to as the **cytopathic effects** (**c.p.e.**) of a virus, and include:

- **Altered shape:** Adherent cells that are normally attached to other cells (*in vivo*) or an artificial substrate (*in vitro*) may assume a rounded shape different from their normal flattened appearance. The extended processes (extensions of the cell surface resembling tendrils) involved in attachment or mobility are withdrawn into the cell.
- **Detachment from the substrate:** For adherent cells, this is the stage of cell damage that follows that just described. Both of these effects are caused by partial degradation or disruption of the cytoskeleton that is normally responsible for maintaining the shape of the cell.
- **Lysis:** This is the most extreme case, where the entire cell breaks down. Membrane integrity is lost, and the cell may swell due to the absorption of extracellular fluid and finally break open. This is an extreme case of cell damage, and it is important to realize that not all viruses induce this effect, although they may cause other cytopathic effects. Lysis is beneficial to a virus in that it provides an obvious method of releasing new virus particles from an infected cell; however, there are alternative ways of achieving this, such as **release** by **budding** (Chapter 4).
- **Membrane fusion:** The membranes of adjacent cells fuse, resulting in a mass of cytoplasm containing more than one nucleus, known as a **syncytium**, or, depending on the number of cells that merge, a giant cell. Fused cells are short lived and subsequently lyse—apart from direct effects of the virus, they cannot tolerate more than one nonsynchronized nucleus per cell.
- **Membrane permeability:** A number of viruses cause an increase in membrane permeability, allowing an influx of extracellular ions such as sodium. Translation of some virus mRNAs is resistant to high concentrations of sodium ions, permitting the expression of virus genes at the expense of cellular messages.

- **Inclusion bodies:** These are areas of the cell where virus components have accumulated. They are frequently sites of virus assembly, and some cellular inclusions consist of crystalline arrays of virus particles. It is not clear how these structures damage the cell, but they are frequently associated with viruses that cause cell lysis, such as herpesviruses and rabies virus.
- **Apoptosis:** Virus infection may trigger apoptosis (programmed cell death), a highly specific mechanism involved in the normal growth and development of organisms (see Chapter 6).

In some cases, a great deal of detail is known about the molecular mechanisms of cell injury. A number of viruses that cause cell lysis exhibit a phenomenon known as **shutoff** early in infection. Shutoff is the sudden and dramatic cessation of most host-cell macromolecular synthesis. In poliovirus-infected cells, shutoff is the result of production of the virus 2A protein. This molecule is a protease that cleaves the p220 component of eIF-4F, a complex of proteins required for cap-dependent translation of messenger RNAs by ribosomes. Because poliovirus RNA does not have a 5′ methylated cap but is modified by the addition of the VPg protein, virus RNA continues to be translated. In poliovirus-infected cells, the dissociation of mRNAs and polyribosomes from the cytoskeleton can be observed, and this is the reason for the inability of the cell to translate its own messages. A few hours after translation ceases, lysis of the cell occurs.

In other cases, cessation of cellular macromolecular synthesis results from a different molecular mechanism. For many viruses, the sequence of events that occurs is not known. In the case of adenoviruses, the penton protein (part of the virus **capsid**) has a toxic effect on cells. Although its precise action on cells is not known, addition of purified penton protein to cultured cells results in their rapid death. Toxin production by pathogenic bacteria is a common phenomenon, but this is the only well-established case of a virus-encoded molecule with a toxin-like action. However, some of the normal contents of cells released on lysis may have toxic effects on other cells, and antigens that are not recognized as self by the body (e.g., nuclear proteins) may result in immune activation and inflammation. The adenovirus E3−11.6K protein is synthesized in small amounts from the E3 promoter at early stages of infection and in large amounts from the major late promoter at late stages of infection (Chapter 5). It has recently been shown that E3−11.6K is required for the lysis of adenovirus-infected cells and the **release** of virus particles from the nucleus.

Membrane **fusion** is the result of virus-encoded proteins required for infection of cells (see Chapter 4), typically, the glycoproteins of **enveloped viruses**. One of the best known examples of such a protein comes from Sendai virus (a paramyxovirus), which has been used to induce cell fusion during the production of monoclonal antibodies (Chapter 1). At least 9 of the 11 known herpes simplex virus (HSV/HHV-1) glycoproteins have been characterized

regarding their role in virus replication. Several of these proteins are involved in fusion of the virus envelope with the cell membrane and also in cell **penetration**. Production of fused **syncytia** is a common feature of HSV infection.

Another virus that causes cell fusion is human immunodeficiency virus (HIV). Infection of $CD4^+$ cells with some but not all isolates of HIV causes cell–cell fusion and the production of syncytia or giant cells (Figure 7.1). The protein responsible for this is the transmembrane envelope glycoprotein of the virus

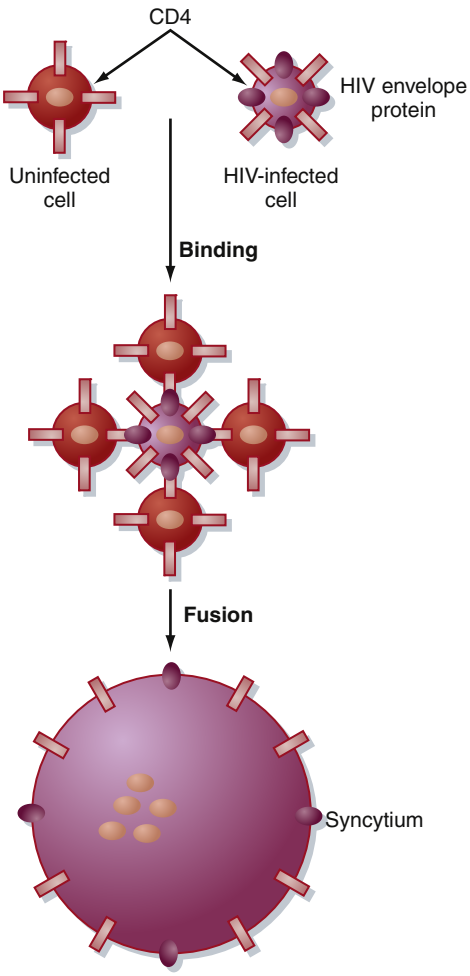

FIGURE 7.1 Mechanism of HIV-induced cell fusion.
The virus envelope glycoprotein, which plays a role on virus particles in receptor binding and membrane fusion, is expressed on the surface of infected cells. Uninfected $CD4^+$ cells coming into contact with these infected cells are fused together to form a multinucleate syncytium.

(gp41), and the domain near the amino-terminus responsible for this fusogenic activity has been identified by molecular genetic analysis. Because HIV infects $CD4^+$ cells and it is the reduction in the number of these crucial cells of the immune system that is the most obvious defect in AIDS, it was initially believed that direct killing of these cells by the virus was the basis for the pathogenesis of AIDS. Although direct cell killing by HIV undoubtedly occurs *in vivo*, it is now believed that the pathogenesis of AIDS is considerably more complex (see "Viruses and Immunodeficiency," next). Many animal retroviruses also cause cell killing and, in most cases, it appears that the envelope protein of the virus is required, although there may be more than one mechanism involved.

VIRUSES AND IMMUNODEFICIENCY

At least two groups of viruses, herpesviruses and retroviruses, directly infect the cells of the immune system. This has important consequences for the outcome of the infection and for the immune system of the host. Herpes simplex virus (HSV) establishes a **systemic infection**, spreading via the bloodstream in association with platelets, but it does not show particular **tropism** for cells of the immune system. However, *Herpes saimirii* and Marek's disease virus are herpesviruses that cause lymphoproliferative diseases (but not clonal tumors) in monkeys and chickens, respectively. The most recently discovered human herpesviruses, human herpesvirus 6 (HHV-6), HHV-7, and HHV-8, all infect lymphocytes (Chapter 8).

Epstein—Barr virus (EBV; HHV-4) infection of B-cells leads to their immortalization and proliferation, resulting in glandular fever or mononucleosis, a debilitating but benign condition. EBV was first identified in a lymphoblastoid cell line derived from Burkitt's lymphoma and, in rare instances, EBV infection may lead to the formation of a malignant tumor (see "Cell Transformation by DNA Viruses," later). While some herpesviruses such as HSV are highly cytopathic, most of the lymphotropic herpesviruses do not cause a significant degree of cellular injury. However, infection of the delicate cells of the immune system may perturb their normal function. Because the immune system is internally regulated by complex networks of interlinking signals, relatively small changes in cellular function can result in its collapse. Alteration of the normal pattern of production of cytokines could have profound effects on immune function. The *trans*-regulatory proteins involved in the control of herpesvirus gene expression may also affect the transcription of cellular genes; therefore, the effects of herpesviruses on immune cells are more complex than just cell killing.

Retroviruses cause a variety of pathogenic conditions including paralysis, arthritis, anemia, and malignant cellular **transformation**. A significant number of retroviruses infect the cells of the immune system. Although these infections may lead to a diverse array of diseases and hematopoetic abnormalities such as

anemia and lymphoproliferation, the most commonly recognized consequence of retrovirus infection is the formation of lymphoid tumors (see "Cell Transformation by RNA Viruses," later). However, some degree of immunodeficiency, ranging from very mild to quite severe, is a common consequence of the interference with the immune system resulting from the presence of a lymphoid or myeloid tumor.

The most prominent aspect of virus-induced immunodeficiency is acquired immunodeficiency syndrome (AIDS), a consequence of infection with human immunodeficiency virus (HIV), a member of the genus *Lentivirus* of the *Retroviridae*. A number of similar lentiviruses cause immunodeficiency diseases in animals. Unlike infection by other types of retrovirus, HIV infection does not directly result in the formation of tumors. Some tumors such as B-cell lymphomas are sometimes seen in AIDS patients, but these are a consequence of the lack of immune surveillance that is responsible for the destruction of tumors in healthy individuals. The clinical course of AIDS is long and very variable. A great number of different abnormalities of the immune system are seen in AIDS. As a result of the biology of lentivirus infections, the pathogenesis of AIDS is highly complex (Figure 7.2).

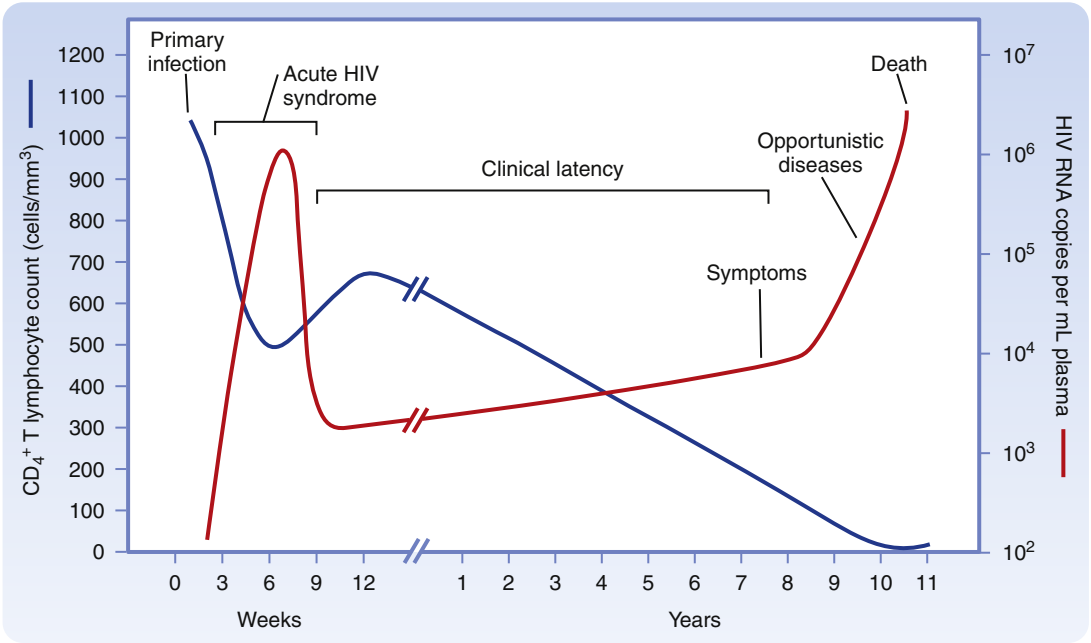

FIGURE 7.2 **Time course of HIV infection.**
This diagram shows a typical sequence of events in an HIV-infected person during the interval between infection with the virus and the development of AIDS.

It is still not clear how much of the pathology of AIDS is caused directly by the virus and how much is caused by the immune system. Numerous models have been suggested to explain how HIV causes immunodeficiency. These mechanisms are not mutually exclusive and indeed it is probable that the underlying loss of $CD4^+$ cells (see Chapter 6) in AIDS is complex and multifactorial. AIDS is defined as the presence of HIV infection, plus one or both of the following:

- A $CD4^+$ T-cell count of less than 200 cells per mL of blood (the normal count is 600 to 1000 per mL)
- Development of an opportunistic infection that occurs when the immune system is not working correctly, such as *Pneumocystis carinii* pneumonia (PCP), certain eye diseases, encephalitis, and some specific tumors such as Kaposi's sarcoma.

The best way to avoid AIDS is not to become infected with HIV, but that's not much help to the 39 million people worldwide who already are infected with the virus. If we are to find a cure for AIDS, we need to understand the mechanisms by which the virus causes the disease. Although the basic biology of HIV is well understood (see "Further Reading" at the end of this chapter), scientists have never had a complete understanding of the processes by which $CD4^+$ T helper cells are depleted in HIV infection, and therefore have never been able to fully explain why HIV destroys the body's supply of these vital cells.

There have been many theories about how HIV infection result in AIDS. Soon after HIV was discovered in the 1980s it was shown that the virus could kill $CD4^+$ cells in culture. Early experiments suggested there might not be enough virus present in AIDS patients to account for all the cell loss seen. More recently, sensitive PCR techniques suggest that with the amount of virus now known to be present in infected individuals, the $CD4^+$ cell count should in fact decline much faster and AIDS develop much earlier than it does after HIV infection. Researchers have used a "tap and drain" analogy to describe $CD4^+$ cell loss in HIV infection. In this description of the disease, $CD4^+$ cells (like water in a sink) are constantly being eliminated by HIV (the drain), while the body is constantly replacing them with new ones (the tap). Over time, the tap cannot keep up with the drain, and $CD4^+$ counts begin to drop, leaving the body susceptible to the infections that define AIDS. $CD4^+$ cells that are activated in response to invading microbes (including HIV itself) are highly susceptible to infection with the virus, and following infection these cells may produce many new copies of HIV before dying.

One explanation for $CD4^+$ cell loss is the "runaway" hypothesis, in which $CD4^+$ cells infected by HIV produce more virus particles, which activate more $CD4^+$ cells that in turn become infected, leading to a positive feedback cycle of $CD4^+$ cell activation, infection, HIV production, and cell destruction.

Unfortunately, mathematical models consisting of a series of equations to describe the processes by which $CD4^+$ cells are produced and eliminated suggest that if the runaway hypothesis was correct, then $CD4^+$ cells in HIV infected individuals would fall to low levels over a few months, not over several years as usually happens. This implies that the runaway hypothesis cannot explain the slow pace of $CD4^+$ cell depletion in HIV infection. That leaves open the question of what exactly is going on between the time someone becomes infected with HIV and the time that they develop AIDS. While virus adaptation (antigenic variation) is important in the biology of HIV, this alone cannot explain the whole story.

In general, HIV is regarded as an incurable infection, although in many cases doctors are able to stave off the onset of AIDS by giving patients sustained courses of antiretrovirus drugs. As a retrovirus, the biology of HIV, including integration of the virus genome into host cell chromosomes, is a major problem in eradicating the virus from the body (see Chapter 3). In HIV-infected people receiving antiviral therapy there is a reservoir of latently infected resting $CD4^+$ T cells. Many HIV patients can manage their infection with cocktails of antiretrovirus drugs that can reduce their viral load—the amount of virus circulating in the blood plasma—to undetectable levels. But even in such noninfectious patients HIV is still lurking in gut tissues, and still infecting other immune cells in the blood. Mathematical modeling and clinical observations suggest it might not ever be possible to completely eradicate the virus from the body with current therapies. The hope is that new approaches such as RNAi (see Chapter 6) might one day be able to tackle this latent virus pool and completely eliminate the virus from the body, curing the infection. Even if this is possible, the cost of these advanced therapies would be beyond the reach of developing countries where the majority of HIV-infected people live. The most important long-term hope for beating HIV infection is therefore to develop effective vaccines to prevent infection, or at least to allow the body to fight the virus more effectively.

BOX 7.2. STEALTHY DOES IT

The more we study viruses, the more examples we find of viruses interacting with the immune system. Not interacting as in "Argh! I'm dead," but interacting as in "I wonder what happens if I twist this knob?" Almost all viruses moderate the immune responses directed against them. This makes sense—if they couldn't do this, they probably wouldn't be able to replicate. And some viruses are masters of the art, subtly tweaking and muting strands of the immune system to make life easier for them. Herpesviruses and poxviruses spring to mind. In comparison to them, viruses that go for an all-out assault on the body or on the immune system seem like amateurs. The consequences on their hosts are devastating, which is bad for both the virus and the host. So let's hear it for the true masters of the craft of sneaking around, of getting on with things quietly.

VIRUS-RELATED DISEASES

Virus infections are believed to be a necessary prerequisite for a number of human diseases that are not directly caused by the virus. In some instances, the link between a particular virus and a pathological condition is well established, but it is clear that the pathogenesis of the disease is complex and also involves the immune system of the host. In other cases, the pathogenic involvement of a particular virus is less certain and, in a few instances, rather speculative.

Although the incidence of measles virus infection has been reduced sharply by **vaccination** (Chapter 6), measles still causes thousands of deaths worldwide each year. The normal course of measles virus infection is an acute febrile illness during which the virus spreads throughout the body, infecting many tissues. The vast majority of people spontaneously recover from the disease without any lasting harm. In rare cases (about 1 in 2000), measles may progress to a severe encephalitis. This is still an acute condition that either regresses or kills the patient within a few weeks; however, there is another, much rarer late consequence of measles virus infection that occurs many months or years after initial infection of the host. This is the condition known as subacute sclerosing panencephalitis (SSPE). Evidence of prior measles virus infection (antibodies or direct detection of the virus) is found in all patients with SSPE, whether they can recall having a symptomatic case of measles or not. In about 1 in 300,000 cases of measles, the virus is not cleared from the body by the immune system but establishes a persistent infection in the CNS. In this condition, virus replication continues at a low level, but defects in the **envelope protein** genes prevent the production of extracellular infectious virus particles. The lack of envelope protein production causes the failure of the immune system to recognize and eliminate infected cells; however, the virus is able to spread directly from cell to cell, bypassing the usual route of infection. It is not known to what extent damage to the cells of the brain is caused directly by virus replication or whether there is any contribution by the immune system to the pathogenesis of SSPE. Vaccination against measles virus and the prevention of primary infection should ultimately eliminate this condition.

Another well-established case where the immune system is implicated in pathogenesis concerns dengue virus infections. Dengue virus is a flavivirus that is transmitted from one human host to another via mosquitoes. The primary infection may be asymptomatic or may result in dengue fever. Dengue fever is normally a self-limited illness from which patients recover after 7 to 10 days without further complications. Following primary infections, patients carry antibodies to the virus. Unfortunately, there are four serotypes of dengue virus (DEN-1, 2, 3, and 4), and the presence of antibody directed against one type does not give cross-protection against the other three; worse still is the fact that antibodies can enhance the infection of peripheral blood mononuclear

cells by Fc-receptor-mediated uptake of antibody-coated dengue virus particles (see Chapter 4).

In a few cases, the consequences of dengue virus infection are much more severe than the usual fever. Dengue hemorrhagic fever (DHF) is a life-threatening disease. In the most extreme cases, so much internal hemorrhaging occurs that hypovolemic shock (dengue shock syndrome, or DSS) occurs. DSS is frequently fatal. The cause of shock in dengue and other hemorrhagic fevers is partly due to the virus, but largely due to immune-mediated damage of virus-infected cells (Figure 7.3). DHF and DSS following primary dengue virus infections occur in approximately 1 in 14,000 and 1 in 500 patients, respectively; however, after secondary dengue virus infections, the incidence of DHF is 1 in 90 and DSS 1 in 50, as cross-reactive but nonneutralizing antibodies to the virus are now present. These figures show the problems of cross-infection with different serotypes of dengue virus, and the difficulties that must be faced in developing a safe **vaccine** against the virus. Dengue virus is discussed further later in this chapter (see "New and Emergent Viruses").

Another instance where virus vaccines have resulted in increased pathology rather than the prevention of disease is the occurrence of postvaccination Reye's syndrome. Reye's syndrome is a neurological condition involving acute cerebral edema and occurs almost exclusively in children. It is well known as

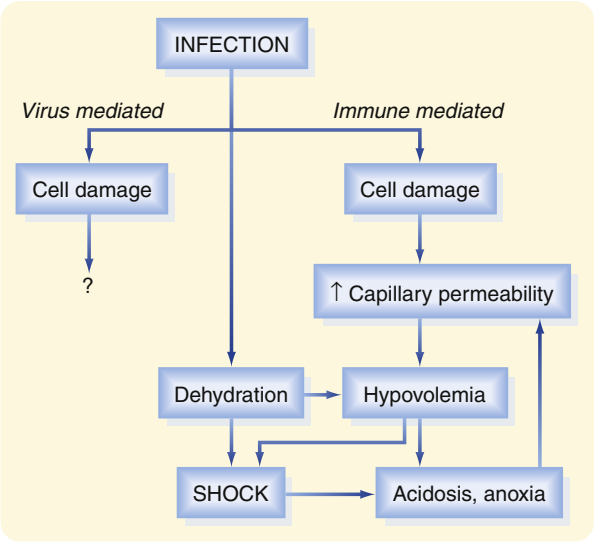

FIGURE 7.3 **Causes of shock in hemorrhagic fevers.**
The cause of hypovolemic shock in dengue and other hemorrhagic fevers is partly due to the virus, but largely due to immune-mediated damage of virus-infected cells.

a rare postinfection complication of a number of different viruses, but most commonly influenza virus and Varicella–Zoster virus (VZV, or chicken pox). Symptoms include frequent vomiting, painful headaches, behavioral changes, extreme tiredness, and disorientation. The chances of contracting Reye's syndrome are increased if aspirin is administered during the initial illness. The basis for the pathogenesis of this condition is completely unknown, but some of the most unfortunate cases have followed the administration of experimental influenza virus vaccines.

Guillain–Barré syndrome is another mysterious condition in which demyelination of nerves results in partial paralysis and muscle weakness. The onset of Guillain–Barré syndrome usually follows an acute virus-like infection, but no single agent has ever been firmly associated with this condition. Kawasaki disease is similar to Reye's syndrome in that it occurs in children but is distinct in that it results in serious damage to the heart. Like Guillain–Barré syndrome, Kawasaki disease appears to follow acute infections. The disease itself is not infectious but does appear to occurs in **epidemics**, which suggests an infectious agent as the cause. A large number of bacterial and virus pathogens have been suggested to be associated with the induction of Kawasaki disease, but once again the underlying cause of the pathology is unknown. It would appear that acute infection itself rather than a particular pathogen may be responsible for the onset of these diseases.

In recent years, there has been a search for an agent responsible for a newly diagnosed disease called chronic fatigue syndrome (CFS) or myalgic encephalomyelitis (ME). Unlike the other conditions described above, CFS is a rather ill-defined disease and is not recognized by all physicians. Recent research has discounted the earlier idea that EBV might cause CFS, but a variety of other possible virus causes, including other herpesviruses, enteroviruses, and retroviruses, have also been suggested. In October 2009 it was reported that 68 of 101 patients with chronic fatigue syndrome (CFS) in the United States were infected with a novel gamma retrovirus, xenotropic murine leukemia virus-related virus (XMRV), a virus previously linked to prostate cancer. This finding, if confirmed, would have a profound effect on the understanding and treatment of an incapacitating disease affecting millions worldwide. Unfortunately, subsequent research findings about XMRV have proved to be contradictory and confusing, and the scientific community is still divided about the role of XMRV in chronic fatigue syndrome.

Some reports have suggested that measles infection before full immunological competence (e.g., younger than 2 years) may be linked to ulcerative colitis and Crohn's disease. This idea is plausible, since measles virus can infect and persist in endothelial cells in the gastrointestinal tract and cause an immune response with giant cell formation; however, we must obtain more evidence

before this can be verified. All these various conditions and syndromes illustrate the complexity of virus pathogenesis and show that the direct effects of virus replication and self-inflicted damage resulting from poor control of the immune system are sometimes difficult to differentiate.

BACTERIOPHAGES AND HUMAN DISEASE

Can **bacteriophages**, viruses that are capable of infecting only **prokaryotic** cells, play a role in human disease? Surprisingly, the answer is yes. Shiga toxin (Stx)-producing *Escherichia coli* (STEC) are able to cause intestinal foodborne diseases such as diarrhea and hemorrhagic colitis. STEC serotype O157:H7, the "hamburger bug," has received much attention in recent years. STEC infections can lead to fatal complications, such as hemolytic–uremic syndrome, as well as neurological disorders. The major virulence characteristics of these strains of bacteria are the ability to colonize the bowel (a natural trait of *E. coli*) and the production of secreted Shiga toxins, which can damage endothelial and tubular cells and may result in acute kidney failure. At least 100 different *E. coli* serotypes produce Stx toxins, and STEC bacteria occur frequently in the bowels of cattle and other domestic animals such as sheep, goats, pigs, and horses. Meat is infected by fecal contamination, usually at the time of slaughter. Ground meat such as hamburger is particularly dangerous as surface bacterial contamination may become buried deep within the meat where it may not be inactivated by cooking.

What has this got to do with bacteriophages? Various types of Stx are known, but they fall into two main types: Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2). The Stx1 and Stx2 toxin genes are encoded in the genome of **lysogenic** lambda-like prophages within the bacteria. Stimuli such as UV light or mitomycin C are known to induce these prophages to release a crop of phage particles that can infect and lysogenize other susceptible bacteria within the gut, accounting for the high prevalence of STEC bacteria (up to 50% of cattle in some herds). Recent research has shown that the scandalous overuse of antibiotics as growth promoters in animal husbandry and even antibiotic treatment of infected people can stimulate the production of phage particles and contribute to the increased prevalence of STEC bacteria and growing human death toll. Other bacterial virulence determinants are also encoded by lysogenic phages (e.g., diphtheria toxin, *Streptococcus* erythrogenic toxins, *Staphylococcus* enterotoxins), although the selective pressures that maintain these arrangements are not yet understood. Emerging bacterial genome sequence data strongly indicate that phages have been responsible for spreading virulence determinants across a wide range of pathogens.

The other area where bacteriophages may influence human illness is phage therapy—the use of bacteriophages as antibiotics. This is not a new idea, with

initial experiments having been performed (unsuccessfully) shortly after the discovery of bacteriophages almost 100 years ago (Appendix 3 WEB); however, with increasing resistance of bacteria to antibiotics and the emergence of superbugs immune to all effective treatments, this idea has experienced a resurgence of interest. Although attractive in theory, this approach suffers from a number of defects:

- Bacteriophages are quite specific in their receptor usage and hence the strains of bacteria they can infect; thus, they are narrow spectrum antibacterial agents.
- Bacteria exposed to bacteriophages rapidly develop resistance to infection by downregulating or mutating the phage receptor.
- Liberation of endotoxin as a consequence of widespread lysis of bacteria within the body can lead to toxic shock.
- Repeated administration of bacteriophages results in an immune response that neutralizes the phage particles before they can act.

It may be, however, that this is a useful therapy for certain bacterial infections that cannot be treated by conventional means. Recently, it has been shown that bioengineered antibodies can be delivered to the brain by bacteriophage vectors, and this novel approach is being investigated for the treatment of Alzheimer's disease and cocaine addiction.

CELL TRANSFORMATION BY VIRUSES

Transformation is a change in the morphological, biochemical, or growth parameters of a cell. Transformation may or may not result in cells able to produce tumors in experimental animals, which is properly known as neoplastic transformation; therefore, transformed cells do not automatically result in the development of cancer. Carcinogenesis (or more properly, oncogenesis) is a complex, multistep process in which cellular transformation may be only the first, although essential, step along the way. Transformed cells have an altered phenotype, which is displayed as one (or more) of the following characteristics:

- **Loss of anchorage dependence:** Normal (i.e., nontransformed) adherent cells such as fibroblasts or epithelial cells require a surface to which they can adhere. In the body, this requirement is supplied by adjacent cells or structures; *in vitro*, it is met by the glass or plastic vessels in which the cells are cultivated. Some transformed cells lose the ability to adhere to solid surfaces and float free (or in clumps) in the culture medium without loss of viability.
- **Loss of contact inhibition:** Normal adherent cells in culture divide and grow until they have coated all the available surface for attachment. At this point, when adjacent cells are touching each other, cell division stops—the cells do not continue to grow and pile up on top of one another. Many

transformed cells have lost this characteristic. Single transformed cells in a culture dish become visible as small thickened areas of growth called transformed foci—clones of cells all derived from a single original cell.

- **Colony formation in semisolid media:** Most normal cells (both adherent and nonadherent cells such as lymphocytes) will not grow in media that are partially solid due to the addition of substances such as agarose or hydroxymethyl cellulose; however, many transformed cells will grow under these conditions, forming colonies since movement of the cells is restricted by the medium.
- **Decreased requirements for growth factors:** All cells require multiple factors for growth. In a broad sense, these include compounds such as ions, vitamins, and hormones that cannot be manufactured by the cell. More specifically, it includes regulatory peptides such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) that regulate the growth of cells. These are potent molecules that have powerful effects on cell growth. Some transformed cells may have decreased or may even have lost their requirement for particular factors. The production by a cell of a growth factor required for its own growth is known as **autocrine** stimulation and is one route by which cells may be transformed.

Cell transformation is a single-hit process; that is, a single virus transforms a single cell (cf. oncogenesis, which is the formation of tumors and a multistep process). All or part of the virus **genome** persists in the transformed cell and is usually (but not always) integrated into the host cell **chromatin**. Transformation is usually accompanied by continued expression of a limited repertoire of virus genes or rarely by **productive infection**. Virus genomes found in transformed cells are frequently replication defective and contain substantial deletions.

Transformation is mediated by proteins encoded by **oncogenes**. These regulatory genes can be grouped in several ways, for example, by their origins, biochemical function, or subcellular locations (Table 7.1). Cell-transforming viruses may have RNA or DNA genomes, but all have at least a DNA stage in their replication cycle; that is, the only RNA viruses directly capable of cell transformation are the retroviruses (Table 7.2). Certain retroviruses carry homologs of c-*oncs* derived originally from the cellular genes and known as v-*oncs*. In contrast, the oncogenes of cell-transforming DNA viruses are unique to the virus genome—there are no homologous sequences present in normal cells. Genes involved in the formation of tumors can be grouped by their biochemical functions:

- **Oncogenes and proto-oncogenes:** Oncogenes are mutated forms of proto-oncogenes, cellular genes whose normal function is to promote the normal growth and division of cells.
- **Tumor suppressor genes:** These genes normally function to inhibit the cell cycle and cell division.

Table 7.1 Categories of Oncogenes

Type	Example
Extracellular growth factors (homologs of normal growth factors)	c-*sis*: Encodes the platelet-derived growth factor (PDGF) B chain (v-*sis* in simian sarcoma virus) *int-2*: Encodes a fibroblast growth factor (FGF)-related growth factor (common site of integration for mouse mammary tumor virus)
Receptor tyrosine kinases (associated with the inner surface of the cell membrane)	c-*fms*: Encodes the colony-stimulating factor 1 (CSF-1) receptor; first identified as a retrovirus oncogene c-*kit*: Encodes the mast cell growth factor receptor
Membrane-associated nonreceptor tyrosine kinases (signal transduction)	c-*src*: v-*src* was the first identified oncogene (Rous sarcoma virus) *lck*: Associated with the CD4 and CD8 antigens of T cells
G-protein-coupled receptors (signal transduction)	*mas*: Encodes the angiotensin receptor
Membrane-associated G-proteins (signal transduction)	c-*ras*: Three different homologs of c-*ras* gene, each identified in a different type of tumor and each transduced by a different retrovirus
Serine/threonine kinases (signal transduction)	c-*raf*: Involved in the signalling pathway; responsible for threonine phosphorylation of mitogen-activated protein (MAP) kinase following receptor activation
Nuclear DNA-binding/transcription factors	c-*myc* (v-*myc* in avian myelocytomatosis virus): Sarcomas caused by disruption of c-*myc* by retroviral integration or chromosomal rearrangements c-*fos* (v-*fos* in feline osteosarcoma virus): Interacts with a second proto-oncogene protein, Jun, to form a transcriptional regulatory complex

- **DNA repair genes:** These genes ensure that each strand of genetic information is accurately copied during cell division of the cell cycle. Mutations in these genes lead to an increase in the frequency of other mutations (e.g., in conditions such as ataxia–telangiectasia and xeroderma pigmentosum).

The function of **oncogene** products depends on their cellular location (Figure 7.4). Several classes of oncogenes are associated with the process of signal transduction—the transfer of information derived from the binding of extracellular ligands to cellular receptors to the nucleus (Figure 7.5). Many of the kinases in these groups have a common type of structure with conserved functional domains representing the hydrophobic transmembrane and hydrophilic intracellular kinase regions (Figure 7.6). These proteins are associated with the cell membranes or are present in the cytoplasm. Other classes of oncogenes located in the nucleus are normally involved with the control of the cell cycle (Figure 7.7). The products of these genes overcome the restriction between the G1 and S phases of the cell cycle, which is the key control point in preventing uncontrolled cell division. Some virus oncogenes

Table 7.2 Cell-Transforming Retroviruses

Virus Type	Time to Tumor Formation	Efficiency of Tumor Formation	Type of Oncogene
Transducing (acutely transforming)	Short (e.g., weeks)	High (up to 100%)	c-*onc* transduced by virus (i.e., v-*onc* present in virus genome; usually replication defective)
cis-Activating (chronic transforming)	Intermediate (e.g., months)	Intermediate	c-*onc* in cell genome activated by provirus insertion; no oncogene present in virus genome (replication competent)
trans-Activating	Long (e.g., years)	Low (<1%)	Activation of cellular genes by *trans*-acting virus proteins (replication competent)

are not sufficient on their own to produce a fully transformed phenotype in cells; however, in some instances, they may cooperate with another oncogene of complementary function to produce a fully transformed phenotype; for example, the adenovirus E1A gene plus either the E1B gene or the c-*ras* gene transforms NIH3T3 cells (a mouse fibroblast cell line). This further underlines the fact that oncogenesis is a complex, multistep process.

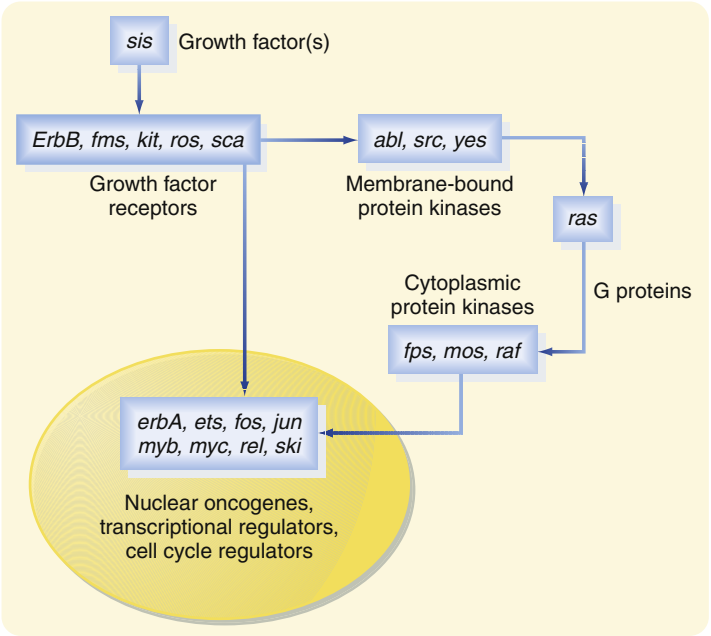

FIGURE 7.4 **Subcellular location of oncoproteins.**
The function of most oncogene products depends on their cellular location (e.g., signal transduction, transcription factors, etc.).

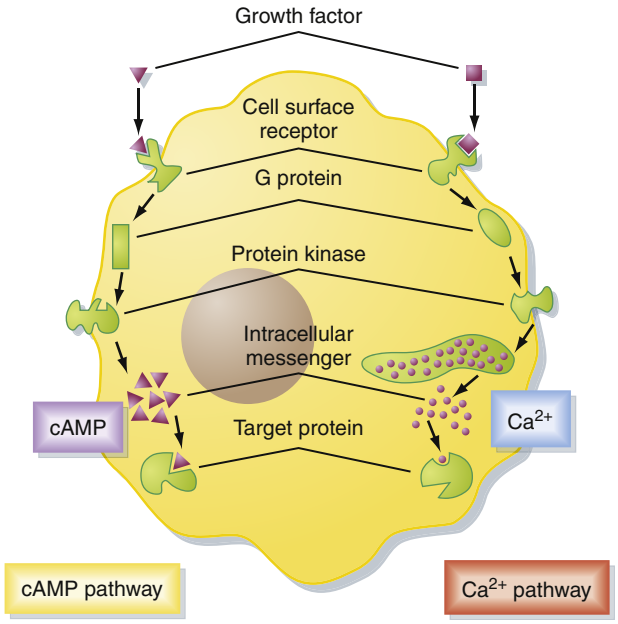

FIGURE 7.5 **Cellular mechanism of signal transduction.**
Several classes of oncogenes are associated with the process of signal transduction, the transfer of information derived from the binding of extracellular ligands to cellular receptors to the nucleus.

CELL TRANSFORMATION BY RETROVIRUSES

Not all retroviruses are capable of transforming cells; for example, lentiviruses such as HIV do not transform cells, although they are cytopathic. The retroviruses that can transform cells fall into three groups: transducing, *cis*-activating, and *trans*-activating. The characteristics of these groups are given in Table 7.2. If **oncogenes** are present in all cells, why does transformation occur as a result of virus infection? The reason is that oncogenes may become activated in one of two ways, either by subtle changes to the normal structure of the gene or by interruption of the normal control of expression. The transforming genes of the acutely transforming retroviruses (v-*oncs*) are derived from and are highly homologous to c-*oncs* and are believed to have been transduced by viruses; however, most v-*oncs* possess slight alterations from their c-*onc* progenitors. Many contain minor sequence alterations that alter the structure and the function of the oncoprotein produced. Others contain short deletions of part of the gene. Most oncoproteins from replication-defective, acutely transforming retroviruses are **fusion proteins**, containing additional sequences derived from virus genes, most commonly virus *gag* sequences at the amino-terminus of the protein. These additional sequences may alter the

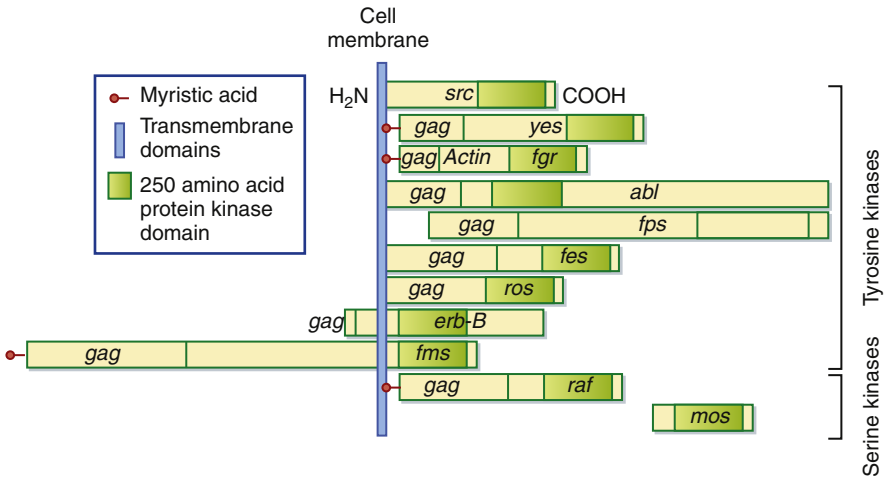

FIGURE 7.6 **Retrovirus protein kinases involved in cell transformation.**
Many of these molecules are fusion proteins containing amino-terminal sequences derived from the *gag* gene of the virus. Most of this type contain the fatty acid myristate, which is added to the *N*-terminus of the protein after translation and which links the protein to the inner surface of the host-cell cytoplasmic membrane. In a number of cases, it has been shown that this posttranslational modification is essential to the transforming action of the protein.

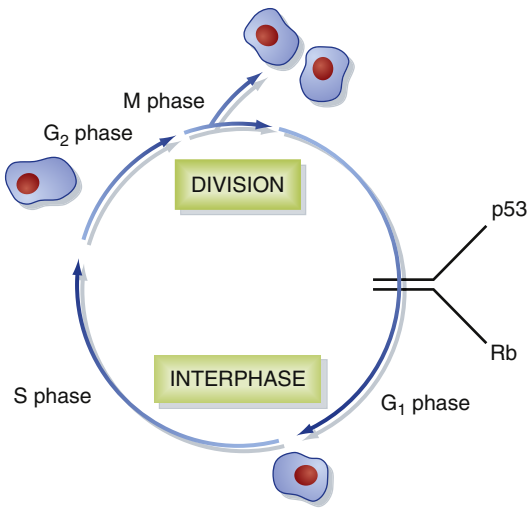

FIGURE 7.7 **Phases of the eukaryotic cell cycle.**
Schematic diagram showing the phases of the eukaryotc cell cycle discussed in the text.

function or the cellular localization of the protein, and these abnormal attributes result in transformation.

Alternatively, viruses may result in abnormal expression of an unaltered oncoprotein. This might be either the overexpression of an oncogene under the control of a virus **promoter** rather than its normal promoter in the cell, or it may be the inappropriate temporal expression of an oncoprotein that disrupts the cell cycle. Chronic transforming retrovirus **genomes** do not contain oncogenes. These viruses activate c-*oncs* by a mechanism known as insertional activation. A **provirus** that integrates into the host-cell genome close to a c-*onc* sequence may indirectly activate the expression of the gene in a way analogous to that in which v-*oncs* have been activated by transduction (Figure 7.8). This can occur if the provirus is integrated upstream of the c-*onc* gene, which might be expressed via a read-through transcript of the virus genome plus downstream sequences; however, insertional activation can also occur when a provirus integrates downstream of a c-*onc* sequence or upstream but in an inverted orientation. In these cases, activation results from **enhancer elements** in the virus promoter (see Chapter 5). These can act even if the provirus integrates at a distance of several kilobases from the c-*onc* gene. The best-known examples of this phenomenon occur in chickens, where insertion of avian leukosis virus (ALV) activates the *myc* gene, and in mice, where mouse mammary tumor virus (MMTV) insertion activates the *int* gene.

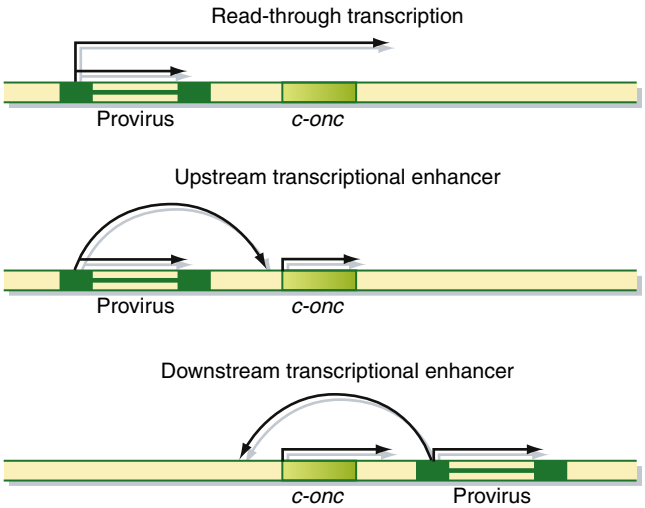

FIGURE 7.8 Transcriptional activation of cellular oncogenes by insertional mutagenesis. Mechanisms by which cellular oncogenes can be transcriptionally activated by retrovirus insertional mutagenesis.

Transformation by the third class of retroviruses operates by quite a different mechanism. Human T-cell leukemia virus (HTLV) and related animal viruses encode a transcriptional activator protein in the virus *tax* gene. The Tax protein acts in *trans* to stimulate transcription from the virus LTR. It is believed that the protein also activates transcription of many cellular genes by interacting with transcription factors (Chapter 5); however, HTLV oncogenesis (i.e., the formation of a leukemic tumor) has a latent period of some 20 to 30 years. Therefore, cell transformation (which can be mimicked *in vitro*) and tumor formation (which cannot) are not one and the same—additional events are required for the development of leukemia. It is thought that chromosomal abnormalities that may occur in the population of HTLV-transformed cells are also required to produce a malignant tumor, although because of the difficulties of studying this lengthy process this is not completely understood.

CELL TRANSFORMATION BY DNA VIRUSES

In contrast to the **oncogenes** of retroviruses, the transforming genes of DNA tumor viruses have no cellular counterparts. Several families of DNA viruses are capable of transforming cells (Table 7.3). In general terms, the functions of their oncoproteins are much less diverse than those encoded by retroviruses. They are mostly nuclear proteins involved in the control of DNA replication, which directly affect the cell cycle. They achieve their effects by interacting with cellular proteins that normally appear to have a negative regulatory role in cell proliferation. Two of the most important cellular proteins involved are known as p53 and Rb.

p53 was originally discovered by virtue of the fact that it forms complexes with SV40 T-antigen. It is now known that it also interacts with other DNA virus oncoproteins, including those of adenoviruses and papillomaviruses. The gene encoding p53 is mutated or altered in the majority of tumors, implying that loss of the normal gene product is associated with the emergence of malignantly transformed cells. Tumor cells, when injected with the native protein *in vitro*, show a decreased rate of cell division and decreased tumorigenicity *in vivo*. **Transgenic** mice that do not possess an intact p53 gene are developmentally

Table 7.3 Transforming Proteins of DNA Tumor Viruses

Virus	Transforming Protein(s)	Cellular Target
Adenoviruses	E1A + E1B	Rb, p53
Polyomaviruses (SV40)	T-antigen	p53, Rb
Papillomaviruses:	E5	PDGF receptor
BPV-1	E6	p53
HPV-16, 18	E7	Rb

normal but are susceptible to the formation of spontaneous tumors; therefore, it is clear that p53 plays a central role in controlling the cell cycle. It is believed to be a tumor suppressor or antioncogene and has been called the "guardian of the genome." p53 is a transcription factor that activates the expression of certain cellular genes, notably WAF1, which encodes a protein that is an inhibitor of G1 cyclin-dependent kinases, causing the cell cycle to arrest at the G1 phase (Figure 7.7). Because these viruses require ongoing cellular DNA replication for their own propagation, this explains why their transforming proteins target p53.

Rb was discovered when it was noticed that the gene that encodes this protein is always damaged or deleted in a tumor of the optic nerve known as retinoblastoma; therefore, the normal function of this gene is also thought to be that of a tumor suppressor. The Rb protein forms complexes with a transcription factor called E2F. This factor is required for the transcription of adenovirus genes, but E2F is also involved in the transcription of cellular genes that drive quiescent cells into S phase. The formation of inactive E2F–Rb complexes thus has the same overall effect as the action of p53—arrest of the cell cycle at G1. Release of E2F by replacement of E2F–Rb complexes with E1A–Rb, T-antigen–RB, or E7–RB complexes therefore stimulates cellular and virus DNA replication.

The SV40 T-antigen is one of the known virus proteins that binds p53. Chapter 5 describes the role of large T-antigen in the regulation of SV40 transcription. Infection of cells by SV40 or other polyomaviruses can result in two possible outcomes:

- Productive (**lytic**) infection
- Nonproductive (**abortive**) infection

The outcome of infection appears to be determined primarily by the cell type infected; for example, mouse polyomavirus establishes a lytic infection of mouse cells but an **abortive infection** of rat or hamster cells, while SV40 shows lytic infection of monkey cells but abortive infection of mouse cells. However, in addition to transcription, T-antigen is also involved in **genome** replication. SV40 DNA replication is initiated by binding of large T-antigen to the origin region of the genome (Figure 5.12). The function of T-antigen is controlled by phosphorylation, which decreases the ability of the protein to bind to the SV40 origin.

The SV40 **genome** is very small and does not encode all the information necessary for DNA replication; therefore, it is essential for the host cell to enter S phase, when cell DNA and the virus genome are replicated together. Protein–protein interactions between T-antigen and DNA polymerase α directly stimulate replication of the virus genome. The precise regions of the T-antigen involved in binding to DNA, DNA polymerase α, p53, and Rb are all

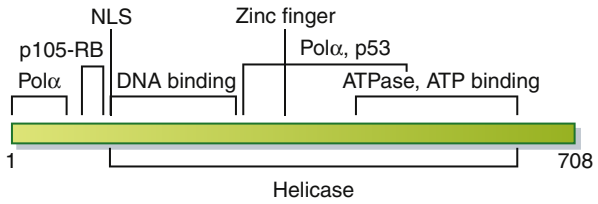

FIGURE 7.9 **Regions of SV40 T-antigen involved in protein–protein interactions.**
Other functional domains of the protein involved in virus DNA replication are also shown, including the helicase, ATPase, and nuclear location signal (NLS) domains.

known (Figure 7.9). Inactivation of tumor suppressor proteins bound to T-antigen causes G1-arrested cells to enter S phase and divide, and this is the mechanism that results in transformation; however, the frequency with which abortively infected cells are transformed is low (about 1×10^{-5}). Therefore, the function of T-antigen is to alter the cellular environment to permit virus DNA replication. Transformation is a rare and accidental consequence of the sequestration of tumor suppressor proteins.

The immediate-early proteins of adenoviruses are analogous in many ways to SV40 T-antigen. E1A is a ***trans*-acting** transcriptional regulator of the adenovirus early genes (see Chapter 5). Like T-antigen, the E1A protein binds to Rb, inactivating the regulatory effect of this protein, permitting virus DNA replication, and accidentally stimulating cellular DNA replication (see earlier). E1B binds p53 and reinforces the effects of E1A. The combined effect of the two proteins can be seen in the phenotype of cells transfected with DNA containing these genes (Table 7.4). However, the interaction of these transforming proteins with the cell is more complex than simple induction of DNA synthesis. Expression of E1A alone causes cells to undergo apoptosis. Expression of E1A and E1B together overcomes this response and permits transformed cells to survive and grow.

Human papillomavirus (HPV) genital infections are very common, occurring in more than 50% of young, sexually active adults, and are usually

Table 7.4 Role of the Adenovirus E1A and E1B Proteins in Cell Transformation

Protein	Cell Phenotype
E1A	Immortalized but morphologically unaltered; not tumorigenic in animals
E1B	Not transformed
E1A + E1B	Immortalized and morphologically altered; tumorigenic in animals

asymptomatic. Certain serotypes of HPV appear to be associated with a low risk of subsequent development of anogenital cancers such as cervical carcinoma, after an incubation period of several decades. 500,000 new cases of cervical neoplasia are diagnosed every year, making this one of the three most common causes of cancer death in women globally. HPV is a primary cause of cervical cancer; 93% of all cervical cancers test positive for one or more high-risk type of HPV. Of the 60 HPV types currently recognized, only four seem to be associated with a high risk of tumor formation (HPV-16, 18, 31, and 45). Once again, transformation is mediated by the early gene products of the virus. However, the transforming proteins appear to vary from one type of papillomavirus to another, as shown in Table 7.3. In general terms, it appears that two or more early proteins often cooperate to give a transformed phenotype. Although some papillomaviruses can transform cells on their own (e.g., BPV-1), others appear to require the cooperation of an activated cellular **oncogene** (e.g., HPV-16/*ras*). In bovine papillomavirus, it is the E5 protein that is responsible for transformation. In HPV-16 and HPV-18, the E6 and E7 proteins are involved.

More confusingly, in most cases all or part of the papillomavirus **genome**, including the putative transforming genes, is maintained in the tumor cells, whereas in some cases (e.g., BPV-4) the virus DNA may be lost after transformation, which may indicate a possible hit-and-run mechanism of transformation. Different papillomaviruses appear to use slightly different mechanisms to achieve genome replication, so cell transformation may proceed via a slightly different route. It is imperative that a better understanding of these processes is obtained. There is no positive evidence that adenoviruses or polyomaviruses are involved in the formation of human tumors. In contrast, the evidence that papillomaviruses are commonly involved in the formation of malignant penile and cervical carcinomas is now very strong.

In recent years, evidence has emerged that p53 and Rb are major cellular sensors for **apoptosis**. Loss of these protein functions triggers apoptosis, the major anticancer mechanism in cells; thus, viruses that interfere with these proteins must have evolved mechanisms to counteract this effect (see discussion in Chapter 6).

VIRUSES AND CANCER

There are numerous examples of viruses that cause tumors in experimental animals, stimulating a long search for viruses that might be the cause of cancer in humans. For many years, this search was unsuccessful, so much so that a few scientists categorically stated that viruses did not cause human tumors. Like all rash statements, this one was wrong. An estimated 20% of all human cancers worldwide may be caused by viruses. Although it is convenient to consider

human tumor viruses as a discrete group of viruses, the six viruses that cause human cancers have very different genomes and replication cycles, and come from six different virus families (HHV-4/EBV, HBV, HCV, HHV-8, HPVs, HTLV). The path from virus infection to tumor formation is slow and inefficient. Only a minority of infected individuals progress to cancer, usually years or even decades after primary infection. Virus infection alone is generally not sufficient for cancer, and additional events and host factors, such as immunosuppression, somatic mutations, genetic predisposition, and exposure to carcinogens must also play a role.

The role of the HTLV Tax protein in leukemia has already been described (see "Cell Transformation by Retroviruses"). The evidence that papillomaviruses may be involved in human tumors is now well established. There are almost certainly many more viruses that cause human tumors, but the remainder of this chapter describes two examples that have been intensively studied: Epstein–Barr virus (EBV) and hepatitis B virus (HBV).

Epstein–Barr virus was first identified in 1964 in a lymphoblastoid cell line derived from an African patient with Burkitt's lymphoma. In 1962, Dennis Burkitt described a highly malignant lymphoma, the distribution of which in Africa paralleled that of malaria. Burkitt recognized that this tumor was rare in India but occurred in Indian children living in Africa and therefore looked for an environmental cause. Initially, he thought that the tumor might be caused by a virus spread by mosquitoes (which is wrong). The association between EBV and Burkitt's lymphoma is not entirely clear cut:

- EBV is widely distributed worldwide but Burkitt's lymphoma is rare.
- EBV is found in many cell types in Burkitt's lymphoma patients, not just in the tumor cells.
- Rare cases of EBV-negative Burkitt's lymphoma are sometimes seen in countries where malaria is not present, suggesting there may be more than one route to this tumor.

Epstein–Barr virus has a dual cell **tropism** for human B-lymphocytes (generally a nonproductive infection) and epithelial cells, in which a **productive infection** occurs. The usual outcome of EBV infection is polyclonal B-cell activation and a benign proliferation of these cells that is frequently asymptomatic but sometimes produces a relatively mild disease known as infectious mononucleosis or glandular fever. In 1968, it was shown that EBV could efficiently transform (i.e., immortalize) human B-lymphocytes *in vitro*. This observation clearly strengthens the case that EBV is involved in the formation of tumors. There is now epidemiological and molecular evidence that EBV infection is associated with at least five human tumors:

- Burkitt's lymphoma.

- Nasopharyngeal carcinoma (NPC), a highly malignant tumor seen most frequently in China. There is a strong association between EBV and NPC. Unlike Burkitt's lymphoma, the virus has been found in all the tumors that have been studied. Environmental factors, such as the consumption of nitrosamines in salted fish, are also believed to be involved in the formation of NPC (cf. the role of malaria in the formation of Burkitt's lymphoma).
- B-cell lymphomas in immunosuppressed individuals (e.g., AIDS patients).
- Some clonal forms of Hodgkin's disease.
- X-linked lymphoproliferative syndrome (XLP), a rare condition usually seen in males where infection with EBV results in a hyperimmune response, sometimes causing a fatal form of glandular fever and sometimes cancer of the lymph nodes. XLP is an inherited defect due to a faulty gene on the X chromosome.

Cellular transformation by EBV is a complex process involving the cooperative interactions between several viral proteins. Three possible explanations for the link between EBV and Burkitt's lymphoma are:

1. EBV immortalizes a large pool of B-lymphocytes; concurrently, malaria causes T-cell immunosuppression. There is thus a large pool of target cells in which a third event (e.g., a chromosomal translocation) results in the formation of a malignantly transformed cell. Most Burkitt's lymphoma tumors contain translocations involving chromosome 8, resulting in activation of the c-*myc* gene, which supports this hypothesis.
2. Malaria results in polyclonal B-cell activation. EBV subsequently immortalizes a cell containing a preexisting c-*myc* translocation. This mechanism would be largely indistinguishable from the preceding.
3. EBV is just a passenger virus. Burkitt's lymphoma also occurs in Europe and North America although it is very rare in these regions; however, 85% of these patients are not infected with EBV, which implies that there are other causes for Burkitt's lymphoma.

Although it has not been formally proved, it seems likely that either (1), (2), or both are the true explanations for the origin of Burkitt's lymphoma.

Another case where a virus appears to be associated with the formation of a human tumor is that of HBV and hepatocellular carcinoma (HCC). Hepatitis is an inflammation of the liver and as such is not a single disease. Because of the central role of the liver in metabolism, many virus infections may involve the liver; however, at least seven viruses seem specifically to infect and damage hepatocytes. No two of these belong to the same family (see Chapter 8). HBV is the prototype member of the family *Hepadnaviridae* and causes the disease formerly known as serum hepatitis. This disease was distinguished clinically from infectious hepatitis (caused by other types of hepatitis virus) in the 1930s. HBV infection formerly was the result of inoculation with human serum

(e.g., blood transfusions, organ transplants) but is still common among intravenous drug abusers, where it is spread by the sharing of needles and syringes; however, the virus is also transmitted sexually, by oral ingestion, and from mother to child, which accounts for familial clusters of HBV infection. All blood, organ, and tissue donations in developed countries are now tested for HBV, and risk of transmission is extremely low. The virus does not replicate in tissue culture, which has seriously hindered investigations into its pathogenesis. HBV infection has three possible outcomes:

1. An acute infection followed by complete recovery and immunity from reinfection (>90% of cases)
2. Fulminant hepatitis, developing quickly and lasting a short time, causing liver failure and a mortality rate of approximately 90% (<1% of cases)
3. Chronic infection, leading to the establishment of a carrier state with virus persistence (about 10% of cases)

There are approximately 350 million chronic HBV carriers worldwide. The total population of the world is approximately 6 billion; therefore, about 5% of the world population is persistently infected with HBV. All of these chronic carriers of the virus are at 100 to 200 times the risk of noncarriers of developing HCC. HCC is a rare tumor in the West, where it represents less than 2% of fatal cancers. Most cases that do occur in the West are alcohol related, and this is an important clue to the pathogenesis of the tumor; however, in Southeast Asia and in China, HCC is the most common fatal cancer, resulting in about half a million deaths every year. The virus might cause the formation of the tumor by three different pathways: direct activation of a cellular oncogene(s), *trans*-activation of a cellular oncogene(s), or indirectly via tissue regeneration (Figure 7.10). As with EBV and Burkitt's lymphoma, the relationship between HBV and HCC is not clear cut:

- Cirrhosis (a hardening of the liver, which may be the result of infections or various toxins, such as alcohol) appears to be a prerequisite for the development of HCC. It would appear that chronic liver damage induces tissue regeneration and that faulty DNA repair mechanisms result eventually in malignant cell transformation. Unrelated viruses that cause chronic active hepatitis, such as the flavivirus hepatitis C virus (HCV), are also associated with HCC after a long latent period.
- A number of cofactors, such as aflatoxins and nitrosamines, can induce HCC-like tumors in experimental animals without virus infection; therefore, such substances may also be involved in human HCC (cf. nitrosamines and NPC, earlier).

For many years, it was thought that HBV integration events were random with regard to their sites within the human genome, but when the relationship between "fragile sites" in the host genome and virus integration events are

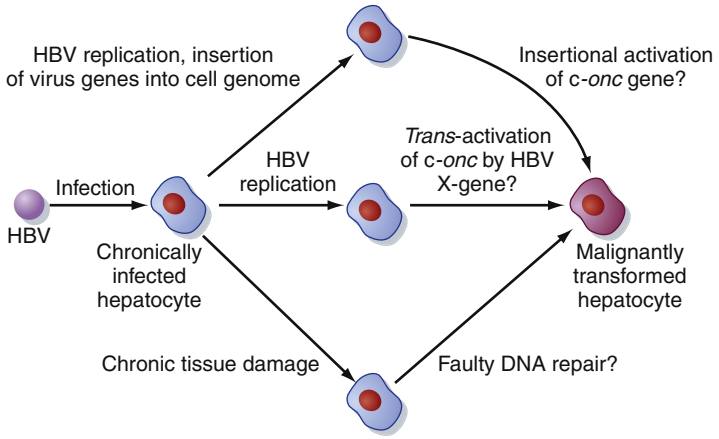

FIGURE 7.10 Possible mechanisms of hepatocellular carcinoma formation due to hepatitis B virus infection.
The complex relationship between HBV infection and HCC means that it is not certain whether any or all of these possible mechanisms are involved.

compared, HBV DNA is found to integrate within or near many of these fragile regions. In most cases, integration at a particular site has been reported for only a single or small number of tumors, but a closer look shows that individual integration sites alter the expression of different components in the same or redundant biochemical or signaling pathways that support hepatocellular growth and survival important for tumor development. Most (but not all) HBV integration events retain the open reading frame encoding the HBx antigen (HBxAg), which suggests that this protein contributes to HCC. It is possible that all the mechanisms shown in Figure 7.10 might operate *in vivo*. The key risk factor is the development of a chronic as opposed to an acute HBV infection. This in itself is determined by a number of other factors:

- Age: The frequency of chronic infections declines with increasing age at the time of infection.
- Sex: For chronic infection, the male:female ratio is 1.5:1; for cirrhosis, the male:female ratio is 3:1.
- HCC: The male:female ratio is 6:1.
- Route of infection: Oral or sexual infections give rise to fewer cases of chronic infection than serum infection.

Until there is a much better understanding of the pathogenesis and normal course of HBV infection, it is unlikely that the reasons for these differences will be understood. There may be a happy ending to this story. A safe and effective

vaccine that prevents HBV infection is now available and widely used in the areas of the world where HBV infection is **endemic** as part of the World Health Organization Expanded Programme on Immunization. This will prevent a million deaths annually from HCC and HBV disease in the future.

NEW AND EMERGENT VIRUSES

What constitutes a new infectious agent? Are these just viruses that have never been discovered, or are they previously known viruses that have changed their behavior? This section will describe and attempt to explain current understanding of a number of agents that meet the previous criteria. Massive and unexpected **epidemics** have been caused by certain viruses. For the most part, these epidemics have not been caused by completely new (i.e., previously unknown) viruses but by viruses that were well known in the geographical areas in which they may currently be causing epidemic outbreaks of disease. Such viruses are known as **emergent viruses** (Table 7.5). There are numerous examples of such viruses that appear to have mysteriously altered their behavior with time, with significant effects on their pathogenesis.

One of the better known examples of this phenomenon is poliovirus. It is known that poliovirus and poliomyelitis have existed in human populations for at least 4000 years. For most of this time, the pattern of disease was **endemic** rather than **epidemic** (i.e., a low, continuous level of infection in particular geographical areas). During the first half the twentieth century, the pattern of occurrence of poliomyelitis in Europe, North America, and Australia changed to an epidemic one, with vast annual outbreaks of infantile paralysis. Although we do not have samples of polioviruses from earlier centuries, the clinical symptoms of the disease give no reason to believe that the virus changed substantially.

Why, then, did the pattern of disease change so dramatically? It is believed that the reason is as follows. In rural communities with primitive sanitation facilities, poliovirus circulated freely. Serological surveys in similar contemporary situations reveal that more than 90% of children of 3 years of age have antibodies to at least one of the three serotypes of poliovirus. (Even the most virulent strains of poliovirus cause 100 to 200 subclinical infections for each case of paralytic poliomyelitis seen.) In such communities, infants experience subclinical immunizing infections while still protected by maternal antibodies—a form of natural **vaccination**. The relatively few cases of paralysis and death that do occur are likely to be overlooked, especially in view of high infant mortality rates.

During the nineteenth century, industrialization and urbanization changed the pattern of poliovirus transmission. Dense urban populations and increased

Table 7.5 Some Examples of Emergent Viruses

Virus	Family	Comments
Cocoa swollen shoot	Badnavirus	Emerged in 1936 and is now the main disease of cocoa in Africa. Deforestation increases population of mealybug vectors and disease transmission.
Hendra virus	Paramyxovirus	Emerged in Brisbane, Australia, September 1994. Causes acute respiratory disease in horses with high mortality and a fatal encephalitis in humans, with several deaths so far. The disease, normally carried by fruit bats (with no pathogenesis), has reemerged in humans in Queensland several times since 1994.
Nipah virus	Paramyxovirus	Emerged in Malaysia in 1998. Closely related to Hendra virus; a zoonotic virus transmitted from animals (pigs?) to humans. Mortality rate in outbreaks of up to 70%.
Phocine distemper	Paramyxovirus	Emerged in 1987 and caused high moralities in seals in the Baltic and North Seas. Similar viruses subsequently recognized as responsible for cetacean (porpoise and dolphin) deaths in Irish Sea and Mediterranean. The virus was believed to have been introduced into immunologically naive seal populations by a massive migration of harp seals from the Barents Sea to northern Europe.
Rabbit hemorrhagic disease (RHD), also known as rabbit calicivirus disease (RCD) or viral hemorrhagic disease (VHD)	Calicivirus	Emerged in farmed rabbits in China in 1984, spread through the United Kingdom, Europe, and Mexico. Introduced to Wardang Island off the coast of South Australia to test potential for rabbit population control, the disease accidentally spread to Australian mainland, causing huge kill in rabbit populations. A vaccine is available to protect domestic and farmed rabbits. In August 1997, RHD was illegally introduced into the South Island of New Zealand, and it escaped into the United States in April 2000.

travelling afforded opportunities for rapid transmission of the virus. In addition, improved sanitation broke the natural pattern of virus transmission. Children were likely to encounter the virus for the first time at a later age and without the protection of maternal antibodies. These children were at far greater risk when they did eventually become infected, and it is believed that these social changes account for the altered pattern of disease.

Fortunately, the widespread use of poliovirus **vaccines** has since brought the situation under control in industrialized countries (Chapter 6). In 1988, WHO committed itself to wiping out polio completely (eradication) by the year 2000. But the disease has proved to be troublingly resilient in a few of the poorest, more corrupt and most dangerous countries, and is still hanging on. Polio eradication is no longer a technical challenge, rather it is a political and economic one.

There are many examples of the **epidemic** spread of viruses caused by movement of human populations. Measles and smallpox were not known to the ancient Greeks. Both of these viruses are maintained by direct person-to-person transmission and have no known alternative hosts; therefore, it has been suggested that it was not until human populations in China and the Roman Empire reached a critical density that these viruses were able to propagate in an epidemic pattern and cause recognizable outbreaks of disease. Before this time, the few cases that did occur could easily have been overlooked.

Smallpox reached Europe from the Far East in 710 AD, and in the eighteenth century it achieved plague proportions—five reigning European monarchs died from smallpox. However, the worst effects occurred when these viruses were transmitted to the New World. Smallpox was (accidentally) transferred to the Americas by Hernando Cortés in 1520. In the next two years, 3.5 million Aztecs died from the disease and the Aztec empire was decimated by disease rather than conquest. Although not as highly pathogenic as smallpox, epidemics of measles subsequently finished off the Aztec and Inca civilizations. More recently, the first contacts with isolated groups of Eskimos and tribes in New Guinea and South America have had similarly devastating results, although on a smaller scale. These historical incidents illustrate the way in which a known virus can suddenly cause illness and death on a catastrophic scale following a change in human behavior.

Measles and smallpox viruses are transmitted exclusively from one human host to another. For viruses with more complex cycles of transmission (e.g., those with secondary hosts and insect vectors), control of infection becomes much more difficult (Figure 7.11). This is particularly true of the families of viruses known collectively as arboviruses (arenaviruses, bunyaviruses, flaviviruses, and togaviruses). As human territory has expanded, this has increasingly brought people into contact with the type of environment where these viruses are found—warm, humid, vegetated areas where insect vectors occur in high densities, such as swamps and jungles.

A classic example is the mortality caused by yellow fever virus during the building of the Panama Canal at the end of the nineteenth century. More recently, the increasing pace of ecological alteration in tropical areas has resulted in the resurgence of yellow fever in Central America, particularly an urban form of the disease transmitted directly from one human to another by mosquitoes. Dengue fever is also primarily an urban disease of the tropics, transmitted by *Aedes aegypti*, a domestic, day-biting mosquito that prefers to feed on humans. Some outbreaks of dengue fever have involved more than a million cases, with attack rates of up to 90% of the population. There are believed to be over 40 million cases of dengue virus infection

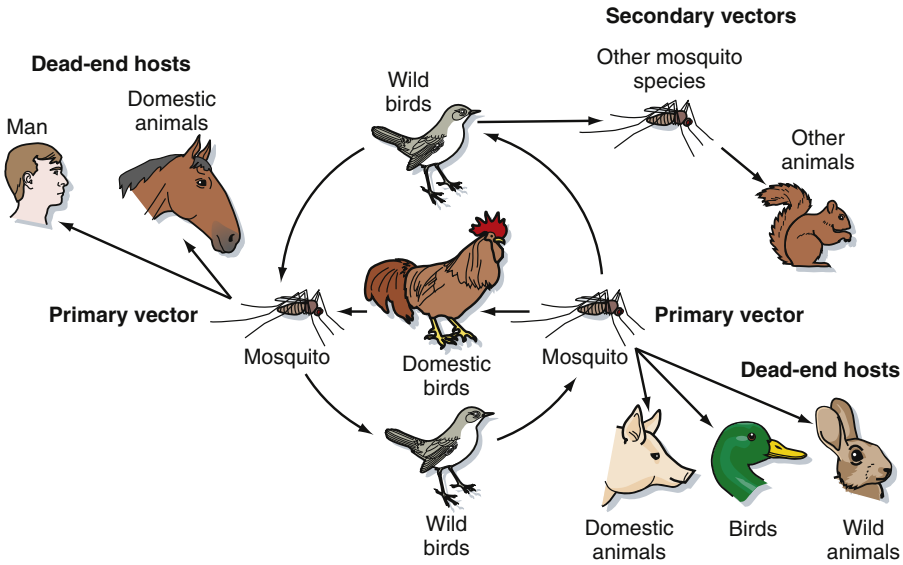

FIGURE 7.11 **Complex transmission pattern of an arbovirus.**
Because of their complex transmission patterns involving multiple host species, arthropod-borne viruses are difficult to control, let alone to eradicate.

worldwide each year. This disease was first described in 1780. By 1906, it was known that the virus was transmitted by mosquitoes, and the virus was isolated in 1944; therefore, this is not a new virus, but the frequency of dengue virus infection has increased dramatically in the last 30 years due to changes in human activity.

Of more than 500 arboviruses known, at least 100 are pathogenic for humans and at least 20 would meet the criteria for **emergent viruses**. Attempts to control these diseases rely on twin approaches involving both the control of insect vectors responsible for transmission of the virus to humans and the development of **vaccines** to protect human populations. However, both of these approaches present considerable difficulties, the former in terms of avoiding environmental damage and the latter in terms of understanding virus pathogenesis and developing appropriate vaccines (see earlier discussion of dengue virus pathogenesis). Rift Valley fever virus (RVFV) was first isolated from sheep in 1930 but has caused repeated **epidemics** in Sub-Saharan Africa during the last few decades, with human infection rates in epidemic areas as high as 35%. This is an epizootic disease, transmitted from sheep to humans by a number of different mosquitoes. The construction of dams that increase mosquito populations, increasing numbers of sheep, and the movement of sheep and human populations are believed to be responsible for the upsurge in

this disease. RVFV continues to extend its range in Africa and the Middle East and is a significant health and economic burden in many areas of Africa, remaining a serious threat to other parts of the world.

The *Hantavirus* genus (*Bunyaviridae*) is a particular cause for concern. Hantaviruses cause millions of cases of hemorrhagic fever each year in many parts of the world. Unlike arboviruses, hantaviruses are transmitted directly from rodent hosts to humans (e.g., via feces) rather than by an invertebrate host. Hantaviruses cause two acute diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). HFRS was first recognized in 1951 after an outbreak among US troops stationed in Korea. In 1993, HPS was first recognized in the United States, and a new virus, Sin Nombre, was identified as the cause. It is now known that at least three different hantaviruses cause HFRS and four different viruses cause HPS. By 1995, HPS had been recognized in 102 patients in 21 states of the United States, in seven patients in Canada, and in three in Brazil, with an overall mortality rate of approximately 40%. These statistics illustrate the disease-causing potential of emerging viruses.

West Nile virus (WNV) is a member of the Japanese encephalitis antigenic complex of the family *Flaviviridae*. All known members of this complex are transmissible by mosquitoes, and many of them can cause febrile, sometimes fatal, illnesses in humans. WNV was first isolated in the West Nile district of Uganda in 1937 but is in fact the most widespread of the flaviviruses, with geographic distribution including Africa and Eurasia. Unexpectedly, an outbreak of human encephalitis caused by WNV occurred in the United States in New York and surrounding states in 1999. In this case, the virus appears to have been transmitted from wild, domestic, and exotic birds by *Culex* mosquitoes (an urban mosquito that flourishes under dry conditions)—a classic pattern of arbovirus transmission. WNV RNA has been detected in overwintering mosquitoes and in birds, and the disease is now endemic across the United States, causing outbreaks each summer. This rapid spread into a new territory shows that spread did not rely on environmental factors such as climate change—the North American environment was already suitable for the virus once it had been introduced, probably via air travel from the Middle East.

Chikungunya virus (CHIKV) is transmitted by *Aedes* mosquitoes and was first isolated in 1953 in Tanzania. CHIKV is a member of the genus *Alphavirus* and the family *Togaviridae*. The disease caused by this virus typically consists of an acute illness characterized by fever, rash, and incapacitating joint pain. The word chikungunya means "to walk bent over" in some east African languages and refers to the effect of the joint pains that characterize this dengue-like infection. Chikungunya is a specifically tropical disease, but was previously

geographically restricted and outbreaks were relatively uncommon. The virus remained largely unknown until a major outbreak in 2005 and 2006 on islands across the Indian Ocean. Plausible explanations for this outbreak (and subsequent spread, which has continued) include increased tourism, CHIKV introduction into a naive population, and virus mutation. It is the last of those three factors that seems to be most significant in this case, with the outbreak strain showing a single amino acid change in the envelope glycoprotein, which allows more effective transmission due to more efficient crossing of the mosquito gut membrane barrier. There is every possibility that CHIKV will continue to extend its territory, with recent outbreaks in Italy.

Plant viruses can also be responsible for **emergent diseases**. Group III geminiviruses are transmitted by insect vectors (whiteflies), and their **genomes** consist of two circular, single-stranded DNA molecules (Chapter 3). These viruses cause a great deal of crop damage in plants such as tomatoes, beans, squash, cassava, and cotton, and their spread may be directly linked to the inadvertent worldwide dissemination of a particular biotype of the whitefly *Bemisia tabaci*. This vector is an indiscriminate feeder, encouraging the rapid and efficient spread of viruses from indigenous plant species to neighboring crops.

Occasionally, there appears an example of an emergent virus that has acquired extra genes and as a result of this new genetic capacity has become capable of infecting new species. A possible example of this phenomenon is seen in tomato spotted wilt virus (TSWV). TSWV is a bunyavirus with a very wide plant host range, infecting over 600 different species from 70 families. In recent decades, this virus has been a major agricultural pest in Asia, the Americas, Europe, and Africa. Its rapid spread has been the result of dissemination of its insect vector (the thrip *Frankinellia occidentalis*) and diseased plant material. TSWV is the type species of the *Tospovirus* genus and has a morphology and genomic organization similar to the other bunyaviruses (Chapter 3). However, TSWV undergoes **propagative transmission**, and it has been suggested that it may have acquired an extra gene in the M segment via **recombination**, either from a plant or from another plant virus. This new gene encodes a **movement protein** (Chapter 6), conferring the capacity to infect plants and cause extensive damage.

In addition to viruses whose ability to infect their host species appears to have changed, new viruses are being discovered continually. After many years of study, three new human herpesviruses have been discovered comparatively recently:

- **Human herpesvirus 6 (HHV-6):** First isolated in 1986 in lymphocytes of patients with lymphoreticular disorders; **tropism** for $CD4^+$

lymphocytes. HHV-6 is now recognized as being an almost universal human infection. Discovery of the virus solved a longstanding mystery: The primary infection in childhood causes roseola infantum or fourth disease, a common childhood rash of previously unknown cause. Antibody **titres** are highest in children and decline with age. The consequences of childhood infection appear to be mild. Primary infections of adults are rare but have more severe consequences—mononucleosis or hepatitis—and infections may be a severe problem in transplant patients.

- **Human herpesvirus 7 (HHV-7):** First isolated from human $CD4^+$ cells in 1990. Its **genome** organization is similar to but distinct from that of HHV-6, and there is limited antigenic cross-reactivity between the two viruses. Currently, there is no clear evidence for the direct involvement of HHV-7 in any human disease, but it might be a cofactor in HHV-6-related syndromes.
- **Human herpesvirus 8 (HHV-8):** In 1995, sequences of a unique herpesvirus were identified in DNA samples from AIDS patients with Kaposi's sarcoma (KS) and in some non-KS tissue samples from AIDS patients. There is a strong correlation (>95%) with KS in both HIV^+ and HIV2 patients. HHV-8 can be isolated from lymphocytes and from tumor tissue and appears to have a less ubiquitous world distribution than other HHVs; that is, it may only be associated with a specific disease state (cf. HSV, EBV). However, the virus is not present in KS-derived cell lines, suggesting that **autocrine** or paracrine factors may be involved in the formation of KS. There is some evidence that HHV-8 may also cause other tumors such as B-cell lymphomas (± EBV as a helper).

Although many different virus infections may involve the liver, at least six viruses seem specifically to infect and damage hepatocytes. No two of these belong to the same family! The identification of these viruses has been a long story:

- Hepatitis B virus (HBV; hepadnavirus): 1963
- Hepatitis A virus (HAV; picornavirus): 1973
- Hepatitis delta virus (HDV; deltavirus; see Chapter 8): 1977
- Hepatitis C virus (HCV; flavivirus): 1989
- Hepatitis E virus (HEV): 1990
- GBV-C/HGV: 1995
- Transfusion-transmitted virus (TTV): 1998

Reports continue to circulate about the existence of other hepatitis viruses. Some of the agents are reported to be sensitive to chloroform (i.e., **enveloped**) while others are not. This may suggest the existence of multiple viruses, as yet

undescribed, although this is still uncertain. New human retroviruses are being discovered regularly, some of them of great significance:

- Human T-lymphotropic virus (HTLV): 1981
- Human immunodeficiency virus (HIV): 1983
- Xenotropic murine leukemia virus-related virus (XMRV): 2006

BOX 7.3. WHERE DO VIRUSES COME FROM?

In spite of what a few people believe, there's no evidence they come from outer space (strike one for the alien abduction theory of virology). So either they come from preexisting viruses that change in some way, or they were there all the time and we just didn't notice them. That's not as stupid as it sounds. Using the molecular clock built into virus genomes researchers have been able to show pretty convincingly that viruses such as measles seemed to pop up just at the point when human populations were big enough to support them by continuous person-to-person spread. And so a cow virus (rinderpest) became a human virus (measles). Like smallpox before them, both measles and rinderpest are now on the verge of complete eradication. But don't get too excited. Just as monkeypox seems to be evolving into the old niche that smallpox filled in Africa, there'll be another virus along to replace measles pretty soon.

ZOONOSES

Many **emergent virus diseases** are **zoonoses** (i.e., transmitted from animals to humans). This emphasizes the importance of the species barrier in preventing transmission of infectious diseases; several recent examples illustrate the potentially disastrous consequences that can occur when this is breached. Strictly speaking, many of the arboviruses discussed earlier are zoonotic in humans, but their transmission involves an insect vector. On occasions, viruses can spread from animals into the human population and then be transmitted from one person to another without the involvement of a vector.

Severe acute respiratory syndrome (SARS) is a type of viral pneumonia, with symptoms including fever, a dry cough, shortness of breath, and headaches. Death may result from progressive respiratory failure due to lung damage. The first SARS outbreak originated in the Guangdong province of China in 2003, where 300 people became ill and at least five died. The cause was found to be a novel coronavirus, SARS-CoV. The SARS virus is believed to be spread by droplets produced by coughing and sneezing, but other routes of infection may also be involved, such as fecal contamination. Where did the SARS virus come from? Coronaviruses with 99% sequence similarity to the surface spike protein of human SARS isolates have been isolated in Guangdong, China, from

apparently healthy masked palm civets, a cat-like mammal closely related to the mongoose. The unlucky palm civet is regarded as a delicacy in Guangdong and it is believed that humans became infected as they raised and slaughtered the animals rather than by consumption of infected meat.

Ebola virus was first identified in 1976. The extreme pathogenicity of this virus has severely inhibited investigations, most of which have been carried out using molecular biological techniques. However, this predominantly molecular approach has left important questions unanswered; for example, some strains of Ebola virus are highly pathogenic, whereas other strains are not. Isolates from Central Africa appear to be highly pathogenic, whereas those from the Philippines are less pathogenic for humans. The molecular basis for these differences is unknown. Most Ebola virus outbreaks appear to be associated with contact with infected primates; however, extensive ecological surveys in Central Africa have failed to show any evidence that primates (or any of the thousands of animals, plants, and invertebrate species examined) are the natural reservoir for infection. No animal reservoir for the virus has been positively identified, but fruit and insectivorous bats support replication and circulation of high titres of Ebola virus without necessarily becoming ill. As with SARS, consumption of exotic wild meats (called bushmeat), particularly primates, may be a risk factor. New zoonotic viruses are frequently discovered, fortunately rarely with the serious disease potential of SARS or Ebola virus.

BIOTERRORISM

Along with the threats from emerging viruses, the world currently faces the potential use of viruses as terrorist weapons. Although this issue has received much media attention, the reality is that the deliberate releases of such pathogens may have less medical impact than is generally appreciated. Many governments devoted considerable resources to the development of viruses as weapons of war before deciding that their military usefulness was very limited. The U.S. Centers for Disease Control (CDC) only recognizes two types of viruses as potentially dangerous terrorist weapons: smallpox and agents causing hemorrhagic fevers such as filoviruses and arenaviruses. Emerging viruses such as Nipah virus and hantaviruses are also recognized as possible future threats. However, this is in contrast to a much larger number of bacterial species and toxins. The reason for this is that bacterial pathogens would be much easier for terrorist groups to prepare and disseminate than viruses. The potential threat from bioterrorism is in reality insignificant in relation to the actual number of deaths caused by infections worldwide each year. Nevertheless, this is an issue that governments are sensibly treating with great seriousness.

SUMMARY

Virus pathogenesis is a complex, variable, and relatively rare state. Like the course of a virus infection, pathogenesis is determined by the balance between host and virus factors. Not all the pathogenic symptoms seen in virus infections are caused directly by the virus—the immune system also plays a part in causing cell and tissue damage. Viruses can transform cells so that they continue to grow indefinitely. In some but not all cases, this can lead to the formation of tumors. There are some well-established cases where certain viruses provoke human tumors and possibly many others that we do not yet understand. The relationship between the virus and the formation of the tumor is not a simple one, but the prevention of infection undoubtedly reduces the risk of tumor formation. New pathogenic viruses are being discovered all the time, and changes in human activities result in the emergence of new or previously unrecognized diseases.

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