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as they are not specific for C. pneumoniae but identify the chlamydiae only to the genus level. TREATMENT C. pneumoniae Infections Although few controlled trials of treatment have been reported, C. pneumoniae is inhibited in vitro by erythromycin, tetracy cline, azithromycin, clarithromycin, gatifloxacin, and gemifloxacin. Directed therapies include azithromycin 500 mg orally once fol lowed by 250 mg on days 2–5; doxycycline 100 mg orally twice daily; or clarithromycin 500 mg twice daily. The fluoroquinolones levofloxacin (750 mg orally once daily) and moxifloxacin (400 mg orally once daily) are alternatives, but their role is limited by increasing safety concerns due to serious side effects. For most patients, a 5-day course of therapy is sufficient. Beta-lactams and trimethoprim/sulfamethoxazole are not active. Acknowledgment The authors wish to thank Dr. Charlotte A. Gaydos for her contributions to this chapter in previous editions. ■ ■FURTHER READING Centers for Disease Control and Prevention: Sexually Trans mitted Infections Surveillance, 2022. Atlanta, GA: U.S. Department of Health and Human Services, 2024. https://www.cdc.gov/std/statistics/2022/ default.htm. Centers for Disease Control and Prevention: Sexually trans mitted infections treatment guidelines, 2021. MMWR Recomm Rep 70:1, 2021. Elwell C et al: Chlamydia cell biology and pathogenesis. Nat Rev Microbiol 14:385, 2016. Gaydos CA, Essiq A: Chlamydiaceae, in Manual of Clinical Microbiol ogy, 11th ed. JH Jorgensen et al (eds). Washington, DC, ASM Press, 2015, pp 1106–1121. Goller JL et al: Population attributable fraction of pelvic inflamma tory disease associated with chlamydia and gonorrhoea: A crosssectional analysis of Australian sexual health clinic data. Sex Transm Infect 92:525, 2016. Gregory ECW, Ely DM: Trends and characteristics of sexually transmitted infections during pregnancy: United States, 2016–2018. National Vital Statistics Reports 69:1, 2020. Hammerschlag MR et al: Chlamydia pneumoniae, in Mandell, Doug las, and Bennett's Principles and Practice of Infectious Diseases, 9th ed. JE Bennett, R Dolin, MJ Blaser (eds). Philadelphia, Elsevier, 2020, Chapter 182. Hughes Y et al: Universal lymphogranuloma venereum (LGV) testing of rectal chlamydia in men who have sex with men and detection of asymptomatic LGV. Sex Transm Infect 98:582, 2022. Kuypers J et al: Principles of laboratory diagnosis of STIs, in Sexu ally Transmitted Diseases, 4th ed. KK Holmes et al (eds). New York, McGraw-Hill, 2008, pp 937–948. Luetkemeyer AF et al: Postexposure doxycycline to prevent bacterial sexually transmitted infections. N Engl J Med 388:1296, 2023. Papp JR et al: Recommendations for the laboratory-based detection of Chlamydia trachomatis and Neisseria gonorrhoeae, 2014. MMWR 63:1, 2014. Rowley J et al: Chlamydia, gonorrhea, trichomoniasis and syphilis: Global prevalence and incidence estimates, 2016. Bull World Health Organ 97:548, 2019. Schachter J, Stephens RS: Biology of Chlamydia trachomatis, in Sexually

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Section 11 Viral Diseases: General Considerations David M. Knipe, Max L. Nibert

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Virology Viruses are obligate intracellular parasites that must enter cells to replicate and propagate themselves to spread to other cells. Infection often injures the host cell—hence the name “virus,” derived from the Latin word virus for poison or toxin. Viruses are one of the simplest life forms and, at the minimum, have a nucleic acid genome with a protein coat. They do not divide by division, as do cells; instead, viruses are programmed to disassemble inside cells, to use their nucleic acid genome to encode viral proteins that replicate their genomic nucleic acid, and then to assemble the progeny genomes into viral particles. The progeny viruses are secreted or released from the host cell as extra cellular virions that infect surrounding cells. Viruses depend on the host cell for many of the enzymes and organelles that synthesize carbo hydrates, lipids, nucleic precursors and nucleic acids, and high-energy molecules, including the host cell’s ribosomes, which are used to make viral proteins. In the process of taking over the host cell, viruses inhibit normal cell metabolic pathways and cause damage to the cell in a pro cess that results in the cytopathic effect (CPE). Injury to cells and cell death can cause tissue damage and contribute to virus-induced disease. CHAPTER 195 Viruses are distinct from other intracellular parasites such as viroids, virusoids, prions, and intracellular bacteria. Viroids are small, circular, single-stranded RNA infectious pathogens of plants that do not have a protein coat, while virusoids are small, circular-RNA, infectious patho gens that depend on viruses to provide the proteins for their replica tion and protein coat. Prions are misfolded proteins that spread from one cell to another, causing the same protein molecules to misfold in the new cell. The misfolded proteins in prions cause cellular damage (Chap. 449). Principles of Medical Virology VIRUS STRUCTURE There are many different virus structures, but nearly all are formed from a few fundamental structural elements. The minimal virion particle is composed of a complex of nucleic acids (the genome) and a protein shell (the capsid) (Fig. 195-1). The combination of the genome and the capsid is called the nucleocapsid. The genome is protected within the capsid. The external surface of virions can consist of either the protein capsid or a lipid envelope around the capsid (Fig. 195-1). Viral genomes can consist of single- or double-stranded RNA or DNA and can comprise one or more genome segments. Singlestranded (ss) genomes are designated as positive strand (+) if they con tain the sequences encoding the open reading frames for viral proteins, while they are designated as negative strand (–) if they contain only complementary sequences. Thus, a positive-strand RNA viral genome can be translated into a viral protein upon entry into the host cell, while a negative-strand genome must be copied into complementary RNA molecules for translation. This dilemma is solved in negative-strand viruses by the loading of transcriptases onto the viral genome prior to encapsidation; these enzymes transcribe the genome into viral mRNA upon entry into and uncoating within the cell. Viral capsids are made of repeating protein subunits because their genomes have limited coding capacity. The capsids are constructed with a few structural units or capsomers packed into a symmetrical arrangement. Capsids are usually organized in one of two ways: (1) an icosahedral or spherical symmetry based on an icosahedron with two-, three-, and fivefold axes of symmetry formed from 20 triangular faces or (2) a helical symmetry. However,

viruses occasionally have more complex structures (e.g., the poxviruses) (Fig. 195-2).

Glycoprotein Genome Genome Capsid Envelope Enveloped virion with icosahedral capsid Nonenveloped icosahedral virion A B FIGURE 195-1 Schematic diagrams of the major forms of human viruses. A. Icosahedral capsid without an envelope. B. Icosahedral capsid with a lipid envelope. C. Helical capsid with a lipid envelope. D. Complex virion. Positive-strand RNA viruses Name Picornaviridae Caliciviridae Hepeviridae Matonaviridae Togaviridae Genome size (kb) 7.5

6.7–10 No Yes Envelope No Caspsid symmetry Icosahedral Icosahedral Icosahedral Negative-strand RNA viruses PART 5 Infectious Diseases Name Rhabdoviridae Filoviridae Genome size (kb) 11–12 15–19 Envelope Yes Yes Caspsid symmetry Helical Helical Segmented negative-strand RNA viruses Segmented double-strand RNA viruses Retroviruses Name Orthomyxoviridae Peribunyaviridae Hantaviridae Nairoviridae Arenaviridae Genome size (kb)

Envelope Yes Yes Yes Caspsid symmetry Helical Helical Helical DNA viruses 100 nm Papillomaviridae Polyomaviridae Parvoviridae Name Hepadnaviridae 5 Kb Genome size 5–9 kbp 3 kbp No No Envelope Caspsid symmetry Icosahedral Icosahedral Icosahedral FIGURE 195-2 Schematic diagrams of viruses of the major families that infect humans. The viruses are grouped by genotype, and the virions are drawn approximately to scale. Prototype viruses of each family are listed in Table 195-1. (Source: Modified from Fig. 185-2 in Harrison’s Principles of Internal Medicine, 20th ed.)

Genome Genome Complex virion Enveloped virion with helical nucleocapsid D C Flaviviridae Coronaviridae 9–13 25–32 Yes Yes Icosahedral Helical Pneumoviridae Paramyxoviridae 14–22 Yes Helical Retroviridae Sedoreoviridae Spinareoviridae 7–13

Yes No Icosahedral Icosahedral Adenoviridae Orthoherpesviridae Poxviridae 36–38 kbp 125–240 kbp 190 kbp Yes No Yes Yes Icosahedral Icosahedral Complex

Enveloped viruses (e.g., measles virus) are efficient in infecting cells because the viral lipid membrane fuses easily with the plasma membrane of the host cell or with internal membranes to deliver the nucleocapsid to the cytoplasm of the host cell. Thus, these viruses are highly transmissible. The lipid envelope is susceptible to disruption by detergents or organic solvents; thus, enveloped viruses such as measles virus, coronaviruses, and influenza viruses can be inactivated by soap and water or alcohol-based hand sanitizers. In contrast, unenveloped viruses (e.g., norovirus or poliovirus) have a tough protein shell whose resistance to small-intestine bile salts—a surfactant that emulsifies lipids—allows them to infect the intestine. Unenveloped viruses, espe cially those that infect the gastrointestinal tract, are not inactivated by detergents or organic solvents and must be inactivated by peroxide or hypochlorite or removed by washing with soap and water. CLASSIFICATION OF VIRUSES Viruses are classified as a free-standing groups because they are not formally related to organisms within any of the major kingdoms. The highest level of viral classification was originally the family, but there have been efforts to classify viruses into higher ranks, culminating in kingdoms and realms. This higher classification is largely not that relevant to medical virology because the major viruses of clinical interest can be conveniently classified into a number of families (Table 195-1), each of which has characteristic virion and genome structures (Fig. 195-2). Classification of viruses into families, genera, and species was

previously based on multiple criteria, including type of genomic nucleic acid (i.e., RNA or DNA; ss positive or negative strand or double strand), capsid symmetry (helical, icosahedral, or complex), presence or absence of an envelope, mode of replication, and tropism (preferred cell type for replication) or type of disease it causes. Recent sequence analysis of viral genomes has refined and revised some of the original virus classifications. The International Committee on Taxonomy of Viruses specifies both formal and common names for viruses. For example, herpes simplex virus (HSV) is the common name for species simplex virus human alpha 1. VIRAL REPLICATION IN CELLS Viral replication takes place in the host cell by the following steps: binding, entry, uncoating, transport to the site of replication, transcrip tion of mRNA, translation of viral proteins, replication of the input genome, assembly of progeny viral particles, and egress from the cell. All viruses must enter cells by mechanisms that allow virus binding to the cell surface and subsequent crossing of the plasma membrane and/ or other membranes to gain entry into the cytoplasm. After entry, the mechanisms of replication diverge for the different viruses, depending on the nature of the viral genome. ■ ■VIRAL ENTRY Viruses bind to specific receptors on the cell surface and generally enter cells by one of three pathways: (1) fusion of the envelope with the surface plasma membrane; (2) endocytosis followed by fusion with the endo some membrane; or (3) lysis of the endosome or formation of pores in the endosome. Viruses often bind to a charged molecule on the surface of cells to concentrate themselves thereon. They then bind more specifically to a protein or carbohydrate molecule, and this binding triggers endocy tosis or fusion of the viral envelope with the cellular plasma membrane. Endocytosis can occur by any of several mechanisms, including clathrinmediated endocytosis, macropinocytosis, micropinocytosis, and caveo lar endocytosis. After viral entry into endocytic vesicles, acidification of the vesicles leads to conformational changes in the viral glycoproteins, fusion of the viral envelope with the endocytic membrane, and release of the nucleocapsid into the cytoplasm. At the entry stage or later, the genome must be uncoated or the capsid opened sufficiently to allow transcription, translation, and/or replication. ■ ■VIRAL REPLICATION STRATEGIES Positive-Strand RNA Viruses The RNA genomes of the picorna viruses, caliciviruses, hepeviruses, togaviruses, flaviviruses, and corona viruses can be translated in the cytoplasm directly after removal of the

capsid coat or uncoating. The picornaviral and flaviviral genomic RNA is translated into a polyprotein that is cleaved by viral and cellular proteases to generate (1) nonstructural proteins that replicate the genomic RNA to complementary negative-strand molecules and then back to positivestrand RNA molecules and (2) structural proteins that assemble capsids for progeny virions. Replication of positive-strand viral RNA takes place in replication complexes associated with cytoplasmic membranes, often in membrane sacs that concentrate the components, protect them from host responses, and provide the redox environment needed for optimal replication. Progeny virions are released when the host cell lyses. The positive-strand genome RNA of the caliciviruses, hepatitis E virus (a hepevirus), and the togaviruses is translated to generate a polyprotein, which, when cleaved by viral and cellular proteases, yields the nonstruc tural proteins that replicate the viral genome to a negative-strand copy and then synthesize new full-length positive strands and a subgenomic mRNA that encodes the structural proteins. Progeny virions are released by budding or cell lysis, depending on whether the virus is enveloped or not. Replication of the genome to the negative strand is followed by a transition back to the positive-strand genome for translation and encap sidation. Progeny virions are released by budding.

Negative-Strand RNA Viruses The rhabdoviruses, filoviruses, and paramyxoviruses have a single negative strand of genome RNA that is transcribed by a virion-associated RNA-dependent RNA poly merase (transcriptase) to yield subgenomic mRNAs that encode the replicase and structural proteins. The replicase copies the full-length negative-strand RNA to a full-length positive-strand RNA and then back to a full-length negative strand, which is assembled into nucleo capsids that bud out of the cell to form progeny virions. CHAPTER 195 The influenza viruses, peri bunyaviruses, and arenaviruses have seg mented negative RNA genomes that are transcribed by virion-associated transcriptases to yield mRNAs that encode nonstructural and struc tural proteins. The replicase enzyme complex copies the negativestrand RNA genomes to full-length positive-strand copies and back to full-length negative-strand RNA molecules. The peri bunyaviruses and arenaviruses replicate entirely in the cytoplasm. In contrast, influenza viral transcription takes place in the nucleus, with nascent cellular transcripts serving as primers to yield mRNAs that are transported to the cytoplasm for translation. Viral proteins are transported into the nucleus to promote genome replication, and progeny negative-strand RNAs are transported to the cytoplasm to bud into progeny virions. Some of the bunyaviruses and the arenaviruses have open reading frames on the “negative strand.” Thus, these viruses use both negative- and positive-sense or ambisense coding of their RNA genomes. The full-length negative strands are assembled in the correct assortment in capsid proteins and then bud to yield infectious progeny virions. Principles of Medical Virology Double-Stranded RNA Viruses The reovirus and rotavirus genomes consist of multiple double-stranded (ds) RNA molecules that are transcribed by virion-associated, RNA-dependent RNA poly merases (transcriptases) to yield mRNAs encoding nonstructural and structural proteins. Following viral protein synthesis, replication of positive-strand RNAs to form dsRNA molecules and assembly into viral capsids occur in cytoplasmic viral factories. Progeny viruses are released when infected cells lyse. Double-Stranded DNA Viruses Most dsDNA viral genomes are transported to the infected cell’s nucleus for transcription and replication. The host cell recognizes foreign DNA that is not fully loaded with histone nucleosomes with a normal pattern and tries to epigenetically silence these molecules; DNA viruses have evolved mechanisms to overcome these epigenetic silencing mechanisms. The dsDNA genomes of the papovaviruses and papillomaviruses are coated with nucleosomal chromatin in the virion and therefore are delivered to the nucleus in a form that is not recognized as foreign. Viral early gene expression is promoted by an enhancer adjacent to the early gene promoter, which is transcribed by host cell RNA polymerase II to yield the early mRNAs. The early proteins promote viral DNA replication by host enzymes, and late genes are then transcribed. The late proteins encode the capsid proteins to assemble progeny virions.

TABLE 195-1 Major Families of Human Pathogenic Viruses FAMILY REPRESENTATIVE VIRUSES TYPE OF RNA/DNA LIPID ENVELOPE Picornaviridae Coxsackievirus Echovirus Enteroviruses, including poliovirus Rhinoviruses Hepatitis A virus Caliciviridae Norovirus (+) RNA No Hepeviridae Hepatitis E virus (+) RNA No Matonaviridae Rubella virus (+) RNA Yes Togaviridae Eastern equine encephalitis virus Western equine encephalitis virus Flaviviridae Yellow fever virus Dengue virus St. Louis encephalitis virus West Nile virus Zika virus Hepatitis C virus Hepatitis G virus Coronaviridae SARS-CoV-1 SARS-CoV-2 Middle East respiratory syndrome virus Rhabdoviridae Rabies virus Vesicular stomatitis virus Filoviridae Marburg virus Ebola virus PART 5 Infectious Diseases Pneumoviridae Respiratory syncytial virus (–) RNA Yes Paramyxoviridae Parainfluenza virus Newcastle disease virus Mumps virus Rubeola (measles) virus Orthomyxoviridae Influenza A, B, and C viruses (–) RNA, 8 segments Yes Peribunyaviridae California encephalitis virus (–) RNA, 3 segments Yes

Hantaviridae Hantavirus (–) RNA, 3 segments Yes Nairoviridae Crimean–Congo hemorrhagic fever virus (–) RNA, 3 segments Yes Arenaviridae Lymphocytic choriomeningitis virus Lassa fever virus South American hemorrhagic fever virus Sedoreoviridae Rotavirus dsRNA, 11 segments No Spinareoviridae Reovirus Colorado tick fever virus Retroviridae Human T lymphotropic virus 1 and 2 Human immunodeficiency virus 1 and 2 Hepadnaviridae Hepatitis B virus dsDNA with ss portions Yes Parvoviridae Parvovirus B19 ssDNA No Papillomaviridae Human papillomaviruses dsDNA No Polyomaviridae JC virus BK virus Merkel cell polyoma virus Adenoviridae Human adenoviruses dsDNA No Orthoherpesviridae Herpes simplex virus 1 and 2 Varicella-zoster virus Epstein-Barr virus Cytomegalovirus Human herpesvirus 6 Human herpesvirus 7 Kaposi's sarcoma–associated herpesvirus Poxviridae Variola (smallpox) virus Orf virus Molluscum contagiosum virus Abbreviations: ds, double-stranded; ss, single-stranded.

(+) RNA No (+) RNA Yes (+) RNA Yes (+) RNA Yes (–) RNA Yes (–) RNA Yes (–) RNA Yes (–) RNA, 2 segments Yes dsRNA, 10–12 segments No (+) RNA, 2 identical segments Yes … … dsDNA Yes dsDNA Yes

The dsDNA genomes of adenoviruses are delivered to the infected cell's nucleus coated with a viral protein that hides the viral genomes from the host's epigenetic silencing mechanisms. Viral DNA genomes are transported to and released through the nuclear pores and are tran scribed by host cell RNA polymerase II to yield pre-early mRNAs. The pre-early proteins promote the transcription of early mRNAs, whose proteins promote viral DNA replication. The late proteins encode structural proteins of the virion. The dsDNA genomes of the herpesviruses, which are not coated with histones in the virion, are transported to the infected cell's nuclear pores and released into the nucleus. The naked DNA is rapidly loaded with histones bearing silencing modifications by host cell mechanisms; however, a viral enhancer and a virion protein that uses host enzymes to drive chromatin reorganization allow immediate-early gene tran scription and expression. Immediate-early proteins promote early gene transcription. Among the E proteins, eight or nine viral proteins including the viral DNA polymerase are essential for viral DNA syn thesis. Late genes then encode proteins for virion assembly. In contrast, the poxviruses replicate entirely in the cytoplasm—an unusual site for replication of a dsDNA virus. As a result, they encode many of the enzymes and factors needed for viral transcription and genome replication. A virus-encoded, virion-associated, DNA-dependent RNA polymerase transcribes the viral genome in the infected cell's cytoplasm to yield early mRNAs. The early mRNAs encode additional transcription factors and DNA replication factors, including a viral DNA polymerase. After DNA replication, the full set of viral proteins needed for viral progeny assembly is generated by intermediate and late transcription. Single-Stranded DNA Viruses The ssDNA genomes of the parvoviruses are delivered to the infected cell's nucleus, and host cell enzymes copy the ssDNA into dsDNA. The dsDNA is then transcribed by the cell's RNA polymerase II to yield mRNAs encoding proteins that promote viral DNA replication and assemble progeny capsids. How the parvoviruses deal with host epigenetic silencing mechanisms is not known. Retroviruses The retrovirus genome consists of two identical positive-strand ssRNA molecules, which are not translated but instead copied into dsDNA by the virion RNA-dependent DNA polymerase or reverse transcriptase upon entry into the host cell's cytoplasm. The dsDNA is transported with the reverse transcriptase–integrase com plex into the nucleus, where the viral integrase catalyzes the integration of the viral DNA molecule into the host cell's chromosomes to yield the provirus. Transcription of the provirus by host RNA polymerase II yields mRNA for translation of viral proteins and for viral full-length transcripts for assembly of progeny virions.

VIRAL EFFECTS ON THE HOST CELL Many viruses inhibit cellular macromolecular processes, such as host cell transcription and protein synthesis, in an attempt to optimize their own replication by usurping the host cell's machinery and bio chemical precursors. These inhibitory events can lead to cell injury and ultimately to cell death, or necrosis. The effects are often manifest by progressive changes in cell structure, detachment from the substrate and rounding up, and eventual lysis. Collectively, these changes are referred to as the CPE. Cells may detect infection as described below and initiate a pathway called programmed cell death, or apoptosis, in an attempt to limit viral infection. Some viruses induce host cell growth to optimize their own replica tion or to amplify the host cells. Papovaviruses, papillomaviruses, and adenoviruses induce the cellular S phase to activate functions needed for viral DNA replication. These viruses also target cellular proteins that control cell growth, inactivating or degrading them to allow the cell cycle to progress to the S phase. Studies of the mechanisms of these viral effects on host cells have identified cellular tumor-suppressor genes such as the p53 and retinoblastoma pRB genes. Epstein-Barr virus induces proliferation to amplify its latent-infection host cell, a B cell. However, the viral mechanisms sometimes induce immor talization of a cell that has already undergone or later undergoes the

oncogenic transformation leading to a cancer cell. Some retroviruses encode altered versions of host genes that can induce transformation. Collectively, these DNA viruses and retroviruses are called tumor viruses.

HOST ANTIVIRAL RESPONSES AND VIRAL ANTAGONISTIC MECHANISMS Host cells have evolved numerous mechanisms for resisting viral infec tion. They encode constitutively expressed proteins that inhibit viral replication in a process called intrinsic resistance. One well-known host resistance factor is the rhesus macaque Trim5α protein, which inhibits human immunodeficiency virus (HIV) type 1 infection soon after the viral core enters the cytoplasm. Viruses have in turn evolved mechanisms by which to evade or neutralize resistance factors in cells of their host species. The promy elocytic leukemia (PML) protein and its associated proteins in nuclear domain 10 (ND-10) structures in the nucleus of human cells restrict HSV replication, but HSV has evolved a gene product—infected cell protein 0 (ICP0), an E3 ubiquitin ligase—that promotes the degra dation of the PML protein and thwarts this antiviral mechanism. Similarly, IFI16 can restrict HSV infection, but the viral ICP0 protein promotes its degradation. Nevertheless, PML and IFI16 protein expres sion are increased by interferon (IFN) signaling, and the elevated levels of these interferon-stimulated genes (ISGs) are sufficient to reduce wild-type HSV infection. Thus, during HSV infection, there is a race between cellular IFN and viral ICP0 expression. ■ ■TYPES OF CELLULAR INFECTIONS The balance of proviral and antiviral factors in a cell defines whether it is permissive or nonpermissive for viral replication. An infection in which progeny virus is produced is a productive infection. If a cell becomes infected but does not die, a virus may establish a persistent infection. A chronic infection can result if infectious virus is continu ally produced. An abortive infection occurs when infection begins but is not completed. In abortive infections, the cell may (1) die, if enough CPEs are exerted, as described above; (2) undergo oncogenic transfor mation; or (3) harbor a latent infection in which no infectious virus is found but the virus can reactivate at a later time. Examples of these outcomes are the abortive oncogenic infection of cells by Merkel cell polyomavirus, chronic infection of liver cells by hepatitis B virus, and latent infection of neurons by HSV. CHAPTER 195 Principles of Medical Virology ■ ■STAGES OF INFECTION OF A HOST The stages of viral infection are (1) entry into the host, (2) primary replication and disease at the site of entry, (3) spread through the host, (4) secondary replication and disease at new sites, (5) persistence or

clearance by the host immune response, and (6) transmission or release from the host. Infection of a host can be acute, chronic, or latent. Entry Keratinized skin cells are not viable and therefore are not good host cells for viral replication. Thus, viruses must enter the host at a mucosal surface (e.g., at oral, respiratory, and nasal sites), through a body opening (e.g., by inhalation or ingestion), or through a break in the skin (e.g., the sites of mosquito or other insect bites). For example, papillomaviruses and HSV enter at breaks in the skin, while Zika and dengue viruses can be introduced via insect bites. Primary Replication and Disease Viruses replicate at the site of entry into the body (i.e., the primary site of infection), are shed back into the environment, and may cause entry-site disease and/or spread to cause systemic illness. For example, influenza viruses can infect the respiratory mucosa. Noroviruses and rotaviruses can infect epithelial cells in the gastrointestinal tract. Dengue and Zika viruses can infect dendritic cells in the tissues after a mosquito bite. If viral infection injures cells and tissues and causes disease at the entry site, the incuba tion period between exposure and disease can be as short as 1 or 2 days. Viral Spread Although some viral infections remain localized at the primary site, others spread from the primary site to secondary sites where the viruses infect new cells and cause disease. This spread may take place through the lymph and the bloodstream (viremia). Measles

virus, for example, replicates initially in the respiratory epithelium, and infected dendritic cells spread through the lymph to lymph nodes where T cells and monocytes are infected and transmit virus through the bloodstream to organs and lymph nodes throughout the body. Systemic disease can result from the disseminated infection, and viral spread into the skin causes the classic measles rash. The incubation period of 10–14 days from exposure to clinical symptoms reflects the time involved for multiple rounds of viral replication and spread within the body before the classic rash symptoms appear. Similarly, dendritic cells and macrophages infected with dengue virus can travel through the circulatory system and transmit virus to secondary sites where infection and disease can follow.

Alternatively, viral spread may occur via neuronal pathways by transsynaptic spread of virions. Rabies virus spreads transsynaptically from the periphery to the central nervous system to cause encephalitis. HSV-1 causes a primary infection at mucosal surfaces and then enters the axon of a sensory neuron and establishes latent infection in the neuron’s cell body. Reactivation usually leads to a recurrent infection at the site of primary infection, but occasionally, the virus can move along nerve tracts to the central nervous system and cause encephalitis. Host Immune Responses Acute viral infection is blunted by the rapid host innate immune response and then controlled by the later adaptive immune response. INNATE IMMUNITY The first arm of the host’s immune response—the innate immune response—is rapid, with recognition of general pat terns of viral molecules but not of specific antigens, whose recognition occurs during the later adaptive response. Using pattern recogni tion receptors, host cells recognize foreign molecules with patterns contained in microbes—i.e., pathogen-associated molecular patterns (PAMPs). Recognition of the foreign molecules leads to activation of innate signaling pathways that induce the expression of IFNs, cyto kines, and other host gene products, including those attributable to IFN-stimulated genes, which serve as antiviral effector molecules. Viral ssRNA is recognized by Toll-like receptor 7 (TLR7) and TLR8, which induce transcription of type I IFN genes and IFN-stimulated genes. IFNs act on the producing cell in an autocrine manner and on sur rounding cells in a paracrine manner to induce expression of antiviral genes and to activate antiviral mechanisms. dsRNA is recognized by TLR3, which activates expression of type I IFNs. ssRNA and dsRNA are recognized by retinoic

acid–inducible gene I (RIG-I) and melanoma differentiation-associated antigen 5 (MDA5), which induce type I IFN expression. Viral glycoproteins are recognized by TLR2 and TLR4. Viral DNA is recognized by the cytoplasmic cGAS receptor, which PART 5 Infectious Diseases Entry Uncoating Synthesis of viral proteins Assembly of progeny virus Copying of viral nucleic acids Release B A FIGURE 195-3 Steps in viral infection of a host cell and effects of immune effector mechanisms. A. Steps in viral infection of a host cell. The steps include entry into the cell, uncoating of the viral genomic nucleic acid, synthesis of viral proteins, copying of viral nucleic acids, assembly of progeny virus, egress, and release from the host cell. B. Mechanisms of immune effector mechanisms. Antibodies can bind to the extracellular virion and neutralize infectivity by preventing binding to the cellular receptor, preventing entry at other steps, preventing uncoating, or preventing other steps of infection. T cells recognize antigenic peptides presented on the surface of infected cells and produce antiviral cytokines and/or activate cell killing.

activates type I IFN expression, and by the nuclear IFN-inducible pro tein 16 (IFI16) receptor, which activates IFN expression in some cell types and epigenetic silencing of the viral DNA genome in many cell types. IFI16 can therefore act as a constitutively expressed resistance factor or as an IFN-stimulated gene. Innate responses also direct the induction of the later, more specific adaptive immune responses. ADAPTIVE IMMUNITY Viral antigens are presented as peptides to both CD4+ and CD8+ T cells by antigen-presenting cells to induce these T cells to develop into antigen-specific T cells. Viral antigens are also presented to B cells, which induce differentiation of antibodyproducing B cells. Antibodies can bind to virions and neutralize their infectivity by preventing their binding to receptors, their entry, their uncoating, or other steps in infection (Fig. 195-3). Antibodies can also bind to viral antigens on the surface of virions and infected cells and promote phagocytosis, antibody-dependent cytotoxicity, and complement-mediated lysis. T cells recognize viral peptides bound to major histocompatibility complex molecules on the surface of infected cells and produce cytokines that exert an antiviral effect or activate cellkilling mechanisms. Thus, the host’s adaptive immune responses can target either virions or infected cells and can clear infection. Long-Term Effects of Infection Persistent infections can lead to continued pathology due to the ongoing immune response, but even acute infection can lead to long-term effects on the host. The chronic sequelae following SARS-CoV-2 infection or long COVID brought attention to this puzzling aspect of viral infection, but this type of viral pathogenesis had been long recognized in various forms of post-acute infection syndromes (PAIS). These included the chronic symptoms following infectious mononucleosis, or acute Epstein-Barr infection, post-dengue fatigue syndrome, and post-polio syndrome, among oth ers. The persisting symptoms may be the result of (1) persisting viral replication or antigens; (2) activation of autoimmune responses; (3) alteration of endogenous bacteria or viruses; and/or (4) irreparable tis sue damage. The large disease burden of long COVID and other forms of PAIS make this a priority for future studies of viral pathogenesis. VIRAL EVOLUTION Because viral RNA-dependent RNA polymerases are error-prone and most do not have editing functions, sequence changes are frequently introduced into their genomes. These alterations can lead to popula tions or swarms of viruses with divergent sequences among a viral population in an individual. Upon drug selection, immune pressure, or host restriction, preexisting variants can emerge as the new major form of a virus. Differences in replicative ability can lead to enrichment Antibody Entry Uncoating Synthesis of viral proteins Assembly of progeny virus Copying of viral nucleic acids T-cell Release Antibody

of more fit viruses and loss of less fit variants. This trend was observed in the COVID-19 pandemic as more fit variants became the dominant forms of SARS-CoV-2 in the population. Viruses with segmented genomes can undergo genome reassort ment in cells co-infected with two viral strains, the result being a new genetic composition for a given virus. For example, new segments can arise in influenza virus isolates thought to be reassortants between the extant human strains and animal or avian strains, such as those from porcine or avian species. This type of event is the cause of the major shifts in influenza viruses that occur periodically over a decade. These major changes due to reassortment and acquisition of a new genome segment are referred to as antigenic shift, as opposed to the small changes due to sequence variation, which are designated antigenic drift. Especially in DNA viruses but—under special circumstances—also in RNA viruses such as coronaviruses, viral genomes can undergo recombination between two strains of virus and generate recombinant genomes with new combinations of genes that may be more or less fit. Viral variants can acquire the ability to infect cells of new host spe cies or to jump species barriers. Zoonotic infection occurs when a virus spreads from animals to humans, as is thought to have occurred with both SARS-CoV-1 and SARS-CoV-2. The original viral ancestor of these viruses—probably endemic in bats—is thought to have spread to other animals sold in the markets of China, and viral variants then arose that could efficiently infect humans. Evolution of variants that could efficiently infect and be transmitted by humans as agents of respiratory infection led to the COVID-19 pandemic. MOLECULAR EPIDEMIOLOGY OF VIRUSES Several molecular techniques allow easy genotyping of virus isolates. Direct sequencing, analysis of polymorphisms in restriction endo nuclease cleavage sites, and polymerase chain reaction (PCR) analysis allow a search for genotypic markers in isolates, with sequencing being the most precise definition of a viral strain. When these types of tests are applied, some viruses (e.g., influenza virus and measles virus) are found to have mainly one strain prevalent in the population at a given time. Thus, only one virus strain spreads through the population. For other viruses, such as HIV or HSV, nearly every unrelated isolate can be differentiated by these tests, and many strains are latent and spread ing within the population and are evolving in parallel. With these molecular techniques, genotypic markers can be used to determine whether a virus has been transmitted from one individual to another. Genomic sequencing studies of SARS-CoV-2 have identified a num ber of major strains circulating at any given time. As new variants have arisen, each has become the dominant circulating strain. DETECTION AND QUANTIFICATION

OF VIRUSES Viruses and viral infections need to be detected and quantified for both clinical and scientific purposes. Diagnostic virology employs the scientific principles described above to detect viruses and evidence of infection in clinical samples, to define the type of virus present in a sample, and in some cases to quantify the amount of virus or the viral load in a patient. Scientific studies use these principles for detection and quantification of viruses in laboratory stocks and for measurement of viral replication. ■ ■DETECTION OF INFECTIOUS VIRUS Biologic assays must be used to detect and measure infectious virus. Infectivity can be measured as either the ability to infect animals and cause disease or the ability to infect cultured cells and cause CPE. For example, SARS-CoV-1 virus was first isolated by the introduction of an oropharyngeal swab sample into Vero cell cultures and detection of CPE. ■ ■DETECTION OF VIRAL PARTICLES, THEIR COMPONENTS, AND VIRAL GENE PRODUCTS Viral Particles Electron microscopy (EM) must be used to visual ize virions directly, because viruses (other than the poxviruses) are smaller than the resolution of the light microscope. Virions can be visualized by EM with negative staining of the virions themselves or by transmission EM of infected cells. As stated above, SARS viral particles

were first visualized in sections of Vero cells infected with samples from patients. The cell culture supernatant showed coronavirus par ticles by negative-staining EM. The latter method has also been used to detect viral particles in stool during outbreaks of gastroenteritis. Antibodies specific for viral capsid proteins are often used in this assay to concentrate the virus and enhance its detection.

Viral Nucleic Acids Viral nucleic acids are detected by amplifica tion methods involving PCR with specific primers, which amplifies very small numbers of viral nucleic acid molecules. These methods can use direct amplification of DNA in clinical samples to detect and quantify viral DNA genomes; alternatively, they can use reverse transcription of RNA followed by PCR to detect a DNA product in clinical samples as a means to detect viral RNA sequences. Multiple primers can be used in a multiplex reaction to detect multiple pathogens. The process of nucleic acid isolation, reverse transcription, and PCR has been automated, and high-throughput instruments measure the HIV load in serum samples. HSV-1 DNA can be measured in cerebrospinal fluid as a rapid assay for HSV encephalitis. These methods have also been transferred to rapid assays for point-of-care detection of viral genomes. Viral Antigens Viral antigens can be detected by immunologic methods such as immunofluorescence and enzyme immunosorbent assay (EIA). Immunofluorescence involves fixation and permeabili zation of cells or tissues from clinical specimens and reaction with either (1) an antiviral antibody conjugated to a fluorophore (direct immunofluorescence) or (2) an antiviral antibody followed by an anti-immunoglobulin antibody conjugated to a fluorophore (indirect immunofluorescence), with detection of the fluorophore by fluores cence microscopy in either case. CHAPTER 195 The EIA entails the immobilization of an antiviral antibody on a substrate such as a microtiter well, incubation of the patient’s sample in the well, and further incubation with an antibody linked to an enzyme. The bound enzyme is then measured by production of a colored sub strate that can be read spectrophotometrically or detected in a rapid antigen test kit. Hemagglutination Some viruses have the ability to cross-link and agglutinate red blood cells of specific species, a process called hemag glutination. Viral titer is measured by the inverse of the last dilution of the sample that causes hemagglutination. Quantitative Assays of Viruses Viruses can be quantified in terms of virion particle numbers and/or infectivity. The number of virion particles in a sample can be determined by negative staining and observation by EM. The numbers of viral DNA genomes can be deter mined by PCR, and RNA genomes can be determined by reverse tran scriptase PCR (RT-PCR), as described above. Alternatively, purified viral particles can be quantified biochemically by spectrophotometric assays that measure viral protein. Principles of Medical Virology The number of infectious particles can be quantified by an endpoint dilution assay in which the virus is diluted until only one-half of cul tures are infected; this concentration is designated the tissue culture infectious dose for 50% of cultures, or TCID50. An alternative assay can determine at what dose one-half of experimental animals die of viral disease (lethal dose for 50% of test animals, or LD50). A more quantita tive assay of infectivity is the plaque assay. A plaque is an area of visual ized localized CPE. In the plaque assay, dilutions of the virus sample are placed on cells attached to a culture dish, and after adsorption of the virus to cells, the cells are overlaid with semisolid medium or medium containing antibody, which prevents virus diffusion through the medium. Virus then spreads only cell to cell, causing a restricted area of CPE—a plaque—on the cellular monolayer. The number of plaques formed by each dilution of virus defines the titer in plaque-forming units (PFUs) per volume of virus stock. For viruses that infect humans, the ratio of viral particles to infec tious units, or the particle-to-PFU ratio, is always much greater than 1—usually 10–1000. This result signifies a large excess of particles that are defective and/or that do not score as infectious in laboratory assays. Thus, for experimental purposes, following

input virus particles, either visually or biochemically, does not guarantee that the observer is

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