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P Miscellaneous Syndromes

130 Chronic Fatigue Syndrome (Systemic Exertion Intolerance Disease)

N. Cary Engleberg

SHORT VIEW SUMMARY

Definition
- As defined in a 1994 Centers for Disease Control and Prevention/National Institutes of Health consensus conference, chronic fatigue syndrome (CFS) is a disorder involving 6 or more months of unexplained, profound fatigue with *at least four* of eight associated symptoms (see Table 130.2).
- A new name, "systemic exertion intolerance disease," was proposed by the Institute of Medicine in 2015, along with somewhat modified diagnostic criteria (see Table 130.3).
- Other names used for this disorder include "postinfectious fatigue," "chronic mononucleosis," "myalgic encephalomyelitis" (United Kingdom/Canada), and "chronic fatigue and immune dysfunction syndrome" (United States).

Epidemiology
- Prevalence is estimated at 0.2% to 1% of the population, depending on the definitions used.
- CFS occurs in women three to seven times more frequently than in men.
- It occurs in all socioeconomic strata and among all races.

Etiology
- CFS may be a sequela of various infectious diseases (e.g., herpesvirus infections, influenza, Q fever, brucellosis, Lyme disease, giardiasis) but is not known to be directly related to any ongoing chronic infection (see Table 130.1).
- Subtle disturbances of immune function are not consistent and not known to be causative of symptoms.
- Hypothalamus-pituitary-adrenal axis or autonomic disturbances may occur, but correcting these subtle anomalies does not improve symptoms of CFS.
- Prior or concurrent history of psychiatric disorders is common.

Diagnosis
- Diagnosis is clinical and usually based on the 1994 consensus definition (see Table 130.2).
- Modified diagnostic criteria have also been proposed by the Institute of Medicine in 2015 (see Table 130.3).
- There is no valid laboratory test to rule in or to rule out CFS.

Therapy
- Pharmacologic therapies are directed at relieving symptoms of pain, sleep disruption, and depression.
- No antimicrobial and immune therapies have been proven to be helpful.
- Nonpharmacologic therapies (cognitive-behavioral therapy and graded exercise therapy) have been repeatedly shown to be helpful in most patients.

Prevention
- There are no known preventive measures.

Chronic fatigue syndrome (CFS) refers to an illness that consists of profound, prolonged fatigue associated with other somatic or neuropsychological symptoms. The diagnosis is based on the patient's subjective report of a compatible symptom cluster in the absence of any medical or psychiatric condition that might account for the complaints. Attempts to ascribe CFS to a single, coherent cause have been fruitless. The available evidence favors the notion that the syndromal definition identifies a heterogeneous population of patients in whom fatigue, pain, cognitive complaints, and viral-like symptoms, such as low-grade fever, sore throat, and tender lymph nodes, are the final common consequences of a variety of different causes. The current, prevailing etiologic hypothesis is that the disorder is a multifactorial condition in which genetic and environmental factors (including infection) interact to produce a disturbed capacity to manage and to control stress, fatigue, and pain.

Other names for this disorder include *chronic fatigue and immune dysfunction syndrome* (or CFIDS) in the United States and *myalgic encephalomyelitis* (or ME) in Great Britain and Canada. Most authorities in the United States preferred the designation *chronic fatigue syndrome* because it is defined by the clinical features of the illness without implication of a specific etiology or characteristic pathology. ME has a much more complex and varied set of diagnostic criteria.[1] However, in recent years combined designations (CFS/ME or ME/CFS) have been used frequently in the literature to accommodate all points of view. A new name, *systemic exertion intolerance disease* (or SEID), has been proposed by the Institute of Medicine (IOM).[2] This new name attempts to capture the central characteristics as well as its severity and complexity with a much simplified diagnostic rubric. *Postviral fatigue* and *postinfectious fatigue* are less strictly defined designations for chronic idiopathic fatigue when the condition is perceived to be induced by an infectious disease and persists after resolution of the infection.

HISTORY

Although popular interest in CFS has been a relatively recent phenomenon, a historical perspective suggests that the illness is not new.[3] For several centuries an illness resembling CFS has been described repeatedly in the medical literature by different names. The illness has been attributed variously to neurologic, cardiovascular, endocrine, and infectious causes. The proximate association of infections, especially influenza, with chronic fatigue (i.e., neurasthenia) was appreciated in the late 19th century.[4] In the 1950s Spink[5] found that nearly 20% of patients with serologic evidence of brucellosis developed lingering symptoms of fatigue, weakness, myalgic pain, mental confusion, and depression in the absence of evidence for continued, active infection, whether or not they had received treatment. He hypothesized that the development of "chronic brucellosis" involved an infection and a psychological predisposition. Later studies by Imboden and coworkers[6] confirmed this impression by showing that patients with chronic brucellosis scored unfavorably on the Minnesota Multiphasic Personality Inventory (MMPI) relative to patients who had recovered from acute brucellosis. To test the hypothesis that a psychological propensity precedes the chronic fatigue illness, these authors conducted a retrospective cohort analysis of military personnel and dependents in Maryland after an outbreak of Asian influenza during the winter of

1957–58.[7] All subjects had completed the MMPI in August 1957, just before the epidemic. Prolonged convalescence from influenza was correlated with preexisting, unfavorable scores on certain subscales of this test. The typical MMPI profile associated with prolonged postinfluenzal symptoms was nearly identical to that observed in patients with chronic brucellosis.

In 1985 two large series of patients with prolonged fatigue and other symptoms were reported to have elevated antibody titers against Epstein-Barr virus (EBV) compared with healthy control subjects.[8,9] In the same year a large outbreak of chronic fatigue with associated symptoms and serologic tests suggesting chronic EBV infection occurred in the area of Lake Tahoe, Nevada.[10] It was proposed that idiopathic chronic fatigue might be due to "chronic mononucleosis." This hypothesis was appealing because it had long been observed that persistent fatigue may follow documented acute mononucleosis in a small proportion of cases.[11–14] However, several subsequent investigations failed to confirm a role for active EBV replication in the persistence of this clinical syndrome.[15–19] Numerous other infectious agents have been proposed as the cause of CFS, including *Candida albicans, Borrelia burgdorferi,* enteroviruses, cytomegalovirus, human herpesvirus 6, spumavirus, retroviruses, *Bornavirus,* and xenotropic murine retrovirus (XMRV) (Table 130.1). The evidence that active infection with any of these agents causes a significant proportion of chronic fatigue cases is either inconclusive or refuted by subsequent investigations.

To stimulate productive research on this clinical problem, the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) sponsored a series of research conferences between 1985 and 1994. One goal of these conferences was to prepare a workable consensus definition of CFS that could be applied uniformly by investigators studying the epidemiology, clinical features, etiology, and treatment of the disorder (Table 130.2). In 2013 the US Department of Health and Human Services, in coordination with a number of other governmental agencies, contracted with the IOM to conduct a study to identify the evidence for various diagnostic criteria for CFS, develop evidence-based clinical criteria, and recommend whether new terminology for CFS should be developed. The IOM's report, published in 2015, proposed somewhat modified diagnostic criteria (Table 130.3) and also proposed that CFS be renamed *systemic exertional intolerance disease.*[2] Before this report there were already a total of eight case criteria defining chronic fatigue illness.[20] The IOM report offers a new diagnostic rubric to unify the field and facilitate timely diagnosis. Better understanding by physicians and the public and less encumbrance of research by multiple case definitions should follow.

EPIDEMIOLOGY

Fatigue is one of the most common complaints encountered in general medical practice. In most patients the complaint is eventually attributed to a diagnosable medical condition or is short lived. According to the most recent consensus conference definition, severe fatigue that remains unexplained after baseline physical and laboratory examinations and persists for more than 6 months is designated as *idiopathic chronic fatigue*. Patients with idiopathic chronic fatigue who also complain of four or more of the associated symptoms listed in Table 130.2 may be considered to have CFS.[21]

Because the case definition requires a medical evaluation, it can be applied only to study the prevalence of CFS in populations who seek medical attention. In a general medical practice in Boston, idiopathic chronic fatigue was reported by 8.5% of patients, but only 0.3% could be diagnosed with CFS.[22] The CDC ascertained the national prevalence of CFS by conducting case finding through a network of physicians in four cities. The prevalence, age, and sex distribution were remarkably similar in the four cities. The prevalence ranged from 3 to 11 per 100,000 population. Patients were predominantly women (7:1) and clustered in the 30- to 50-year age group.[23] Similar estimates of prevalence were reported from studies in Australia and the United Kingdom.[24,25] The characteristic predominance of upper-middle-class white women in their 30s and 40s resulted in the pejorative term "yuppie flu." These and other observations are biased, however, by reliance on clinic-based case ascertainment. When a random telephone survey was conducted in the San Francisco area, a different epidemiologic pattern emerged.[26] The

TABLE 130.1 Proposed Infectious Causes of Chronic Fatigue Syndrome

PROPOSED ETIOLOGIC AGENT	REFERENCES: Suggestive Studies	REFERENCES: Negative Studies
Epstein-Barr virus	8, 9, 187	15, 17, 18, 48, 188, 189
Cytomegalovirus	190, 191	15, 48, 188, 189
Human herpesvirus 6	49, 50, 192, 193	15, 48, 194, 195, 189
Human herpesvirus 7	183, 196	50, 189, 192, 195
Human herpesvirus 8	—	50, 189, 197
Enteroviruses	198–200	15, 48, 201–204
Parvovirus	43, 205	206, 207, 189
GB virus-C		208
Human spumavirus	209	—
Bornavirus	210, 211	212
Human retrovirus	213	214–216
Xenotropic murine retrovirus (XMRV)	49, 50	54, 56–58 59 (retraction of 49) 60 (retraction of 50)
Borrelia burgdorferi	217	218, 219
Brucella spp.	220	5
Mycoplasma spp.	221, 222	223
Candida albicans	224, 225	226

TABLE 130.2 CDC/NIH Consensus Conference Definition of Chronic Fatigue Syndrome

Clinically evaluated, unexplained chronic fatigue for >6-mo duration, which is not lifelong or the result of ongoing exertion and is not alleviated substantially by rest. Fatigue is associated with a significant reduction in occupational, educational, social, or personal activities *plus* ≥4 of the following concfurrent symptoms:

- Impaired memory or concentration
- Sore throat
- Tender cervical or axillary lymph nodes
- Muscle pain
- Multijoint pain
- New headaches
- Unrefreshing sleep
- Postexertional malaise

CDC, Centers for Disease Control and Prevention; *NIH,* National Institutes of Health.

Modified from Fukuda K, Straus SE, Hickie I, et al. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med. *1994;121:953–959.*

TABLE 130.3 IOM Diagnostic criteria for Systemic Exertion Intolerance Disease

Diagnosis requires that the patient have the following three symptoms:

1. A substantial reduction or impairment in the ability to engage in preillness levels of occupational, educational, social, or personal activities; that persists for more than 6 months and is accompanied by fatigue, which is often profound; is of new or definite onset (not lifelong); is not the result of ongoing excessive exertion, and is not substantially alleviated by rest *and*
2. Postexertional malaise[a] *and*
3. Unrefreshing sleep[a]

At least one of the two following manifestations is also required:

1. Cognitive impairment[a] *or*
2. Orthostatic intolerance

[a]Frequency and severity of symptoms should be assessed. The diagnosis of myalgic encephalomyelitis/chronic fatigue syndrome should be questioned if patients do not have these symptoms at least half of the time with moderate, substantial, or severe intensity.

IOM, Institute of Medicine.

From Institute of Medicine. Beyond Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Redefining an Illness. *Washington, DC: National Academies Press; 2015.*

overall prevalence of subjects reporting a CFS-like illness was 0.2% of the population, and the female predominance was less dramatic (2.9:1). The age distribution was the same as that seen in clinic-based studies, but the distribution of cases by income showed higher rates in persons with family incomes less than $40,000, suggesting that the perception of "yuppie flu" is an artifact of health care use by the affected populations. The rates of CFS-like illness were higher in African Americans and Hispanics than in whites and Asian Americans, and there was no preponderance of cases in any individual occupational group. Similar findings have been reported in other community-based studies.[27,28] These studies suggest that there is a high frequency of chronic fatigue in all communities; however, review of individual medical records reveals a medical or psychiatric condition to account for a majority of the cases. In addition, the prevalence of idiopathic chronic fatigue (usually about 1%–6%) is much greater than the number that meets the CDC consensus criteria for CFS (0.1%–0.3%).

Investigators in the United Kingdom used a population-based birth cohort database to estimate the occurrence of "chronic disabling fatigue" (CDF) in adolescents, defined by fatigue of ≥6 months duration, but not necessarily meeting CFS criteria. At the age of the 13, the point prevalence was 1.3%,[29] and there was an association with a family adversity score. A subsequent analysis drawn from the same database found a point prevalence of 1.9% at age 16.[30] After excluding subjects with severe depression, the prevalence of CDF dropped to 0.6%. Of interest, female gender comparable to that seen in adults appeared among 16-year-olds but not 13-year-olds. A longitudinal assessment of CDF in this population showed an increasing prevalence between ages 13 and 18 years; however, 75% recover within 2 to 3 years.[31]

Outbreaks of idiopathic illness consistent with CFS have been reported occasionally since the 1940s.[32] In many of these outbreaks the involvement of an infectious agent is unlikely because certain subgroups of the population at risk were affected disproportionately. Large hospital outbreaks in Los Angeles and London affected the professional staff but not the hospitalized patients or nonprofessional staff.[33,34] An acute outbreak of neuromyasthenia in New Zealand resulted in CFS in many of the affected individuals. A 10-year follow-up of these cases indicated that most patients had recovered partially or completely.[35]

ETIOLOGY AND PATHOGENESIS

Attempts to elucidate the pathophysiology of CFS have been hampered by several methodologic problems. Foremost among these is the problem of selecting a homogeneous group of subjects for study from among patients identified by the working definition. In a symptom cluster analysis of patients meeting the CDC criteria, at least two distinct subgroups emerged: one having numerous syndromal and nonsyndromal symptoms with high severity scores, and a second, larger group with limited symptoms and only moderate severity.[36] A recent genetic and clinical symptom analysis suggests the existence of seven discrete subgroups.[37]

It has long been appreciated that some patients have an acute onset associated with an infectious disease or other definable stressor, whereas others describe an insidious and progressive onset. Most patients have past or current psychiatric disorders,[38–40] whereas some have no past or present psychiatric symptoms. There is no reason to assume a priori that patients with these diverse clinical circumstances have the same disorders simply because they meet the CDC criteria at the time of presentation.

Role of Infection

Infectious causes of CFS have been proposed (see Table 130.1), but there is no reproducible evidence that any single agent is responsible for any significant proportion of these illnesses. Postinfectious fatigue cases indistinguishable from CFS may immediately follow a diagnosed infection (e.g., EBV infection, influenza, brucellosis, giardiasis), including some that are highly localized geographically (e.g., Lyme disease, Q fever, Ross River virus infection).[7,41–44] In a prospective study Hickie and coworkers[14] reported that postinfectious fatigue occurs at a rate of approximately 12% whether the instigating infection is EBV, Q fever, or Ross River virus. Most of these patients met criteria for CFS as well. Others have reported that postinfectious and idiopathic cases have indistinguishable clinical and psychosocial features.[45] Prolonged convalescence from infectious mononucleosis is a well-recognized phenomenon.[11–13] A more recent prospective study of adolescents with infectious mononucleosis showed that at 6 and 24 weeks of follow-up, 13% and 4%, respectively, had prolonged fatigue that met CDC criteria.[46] The use of steroids during the acute phase of mononucleosis was not associated with prolonged postinfectious fatigue in this study.

The occurrence of clinically indistinguishable "postinfectious" fatigue states after several unrelated infections supports the concept that CFS is a nonspecific sequela to a variety of illnesses rather than a disease with a single, specific infectious cause. Controversy persists about whether chronic fatigue can be triggered by any infectious or traumatic event or whether only a particular type of infection or trauma is necessary. However, patients seen in general practice for common infections do not have an increased frequency of prolonged fatigue relative to patients seen for other medical problems.[47]

Although there was never any direct virologic evidence favoring chronic, persistent EBV infection as a cause of CFS, a significant body of negative research has accumulated to reject this hypothesis. These studies showed no significant differences in serologic titers,[15,48] shedding of virus in saliva,[19] blood lymphocyte-transforming activity,[19,18] or EBV-specific cytotoxic lymphocytes[18] between patients and healthy control subjects. In addition, a large treatment study compared intravenous and oral acyclovir with placebo in CFS patients and failed to show any benefit.[17] Similarly, negative microbiologic data have been collected for other specific infectious agents (see Table 130.1). The possibility that CFS patients may reactivate latent viruses more frequently than healthy individuals has been proposed,[49,50] but it is not clear how or whether viral reactivation affects the ongoing symptom complex.[51] For example, Chia and Chia[52] have recently observed that enterovirus RNA and capsid protein VP1 are found four times more frequently in the stomachs of CFS patients than in healthy control subjects. However, like many similar virologic observations, it is not clear whether persistent enterovirus is a cause or a consequence of the syndrome.

A high-profile controversy concerning an infectious etiology for CFS occurred after a 2009 publication implicating XMRV in 67% of 101 patients but only 3.7% of 218 healthy control subjects.[53] A separate research group reported a similar disparity in the frequency of murine leukemia virus–related sequences among CFS patients and healthy blood donors.[54] Initially, criticism of these reports from established researchers focused on poorly described selection of cases and control subjects, potential sources of bias, and uncertainty about the generalizability of the findings.[55–57] However, the most serious challenge came from DNA sequence analysis showing that viruses in an established murine cell line are ancestral to viruses detected in the human samples, suggesting that the human samples were contaminated with mouse DNA.[58] Several subsequent studies failed to show any association of XMRV with CFS,[59–62] and the two studies that initially found an association were eventually retracted (see Table 130.1).[54,63]

Role of the Autonomic Nervous System

In 1995 researchers at Johns Hopkins University reported that chronic fatigue patients had abnormal responses to a 45-minute tilt-table test protocol.[64] Virtually all of the patients had syncope or reproduction of fatigue symptoms, whereas only about one-third of healthy control subjects had an abnormal response. These investigators proposed that the persistent fatigue may be attributable to neurally mediated hypotension. Subsequent studies have provided mounting evidence that younger patients with CFS have increased sympathetic activity at rest with exaggerated cardiovascular response to orthostatic stress. Resting catecholamine levels are increased, and thermoregulatory responses suggest increased sympathetic activation at rest.[65,66] This may be manifest as positional orthostatic tachycardia syndrome without hypotension.[67] However, Katz and coworkers[68] failed to find any difference in the frequency of orthostatic intolerance among adolescents 6 months after infectious mononucleosis, whether they met criteria for CFS or had completely recovered.

Attempts to treat CFS as neurally mediated hypotension have not been successful. The investigators who demonstrated abnormal tilt-table

testing in young patients with CFS used combinations of fludrocortisone, atenolol, and sertraline with increased salt intake to treat a cohort of patients. Initially, they reported a favorable durable response in 47% of 19 patients.[64] However, two subsequent controlled trials failed to show any benefit using this approach to therapy.[69,70]

Role of the Immune System

Some of the proposed inciting and perpetuating factors involve disruptions in various biologic systems (e.g., the immune system, skeletal muscle, heart, CNS). Although subtle alterations in some of these systems have been identified in patients, similar changes are observed in individuals without symptoms. Differences in various measures of immune function (e.g., cytokine levels, in vitro lymphocyte function, flow cytometry) have been observed by comparing CFS patients and healthy persons; however, many of the studies are inconsistent with one another or have not been reproduced. One prospective study of postinfectious fatigue failed to find any significant differences in the levels of eight cytokines comparing patients and control subjects.[71] In another study alterations in cytokine profiles were only found in patients with a disease duration of less than 3 years.[72]

The most consistent immune alterations have included an increase in T lymphocytes expressing activation markers,[73] for instance, in the number of lymphocytes bearing the CD45RA differentiation marker.[74] These findings suggest mild activation of the cellular immune system, but the relevance of these alterations is unclear, given the inconsistent immunologic differences among monozygotic twin pairs that are discordant for CFS.[75] It is also not clear that a change in lymphocyte subsets occurs in conjunction with clinical improvement.[76]

Another immunologic finding more common in CFS patients than in healthy control subjects is a reduction in natural killer cell function and in the number of lymphocytes bearing the CD16 marker.[77,78] This finding raised the concern that malignancies might occur at an increased rate in CFS. Analysis of cancer registries after the large 1985 Lake Tahoe outbreak did not support this notion.[79] Subsequently, Chang and coworkers[80] queried the Surveillance, Epidemiology, and End Results (SEER)-Medicare registry that includes 1.2 million cancer cases and compared them with 100,000 control subjects. The frequency of a CFS diagnosis more than 1 year before selection was 0.5% in both the cancer and control groups; however, an extensive subgroup analysis showed a small increase in the odds of CFS in patients with non-Hodgkin lymphoma. Two studies of all-cause mortality showed no increased rates for CFS patients.[81,82]

Abnormalities of B lymphocytes have been present less consistently among CFS patients. However, a Norwegian research group reported a transient beneficial effect of rituximab therapy in a majority of 15 treated CFS patients compared with 15 placebo-treated control subjects.[83] This observation has been followed up with an open-label, one-armed (uncontrolled) phase II study suggesting clinical response during the period of B-cell depletion.[84]

Dysregulation of RNase L has been studied as a common feature of CFS. Normally, the presence of double-stranded (ds)RNA (i.e., from viruses) activates expression of the enzyme 2′,5′-oligoadenylate synthetase. The 2′,5′-adenylate oligonucleotides generated from this activity bind to and activate RNase L, which degrades viral and some host RNAs. CFS patients are purported to have increased levels of 2′,5′-adenylate oligonucleotides and RNase L activity.[85,86] In addition, a low-molecular-weight RNase L molecule has been detected more frequently in CFS patients than in healthy control subjects (88% vs. 32%).[87,88] Activation of this pathway in CFS could be regarded as evidence of a response to viral infection or, alternatively, as evidence of disturbed immune regulation that results in unnecessary degradation of host RNA.

A synthetic dsRNA rintatolimod (Ampligen; Hemispherx Biopharma, New Brunswick, NJ) activates 2′,5′-oligoadenylate synthetase via Toll-like receptor 3 (TLR-3) and generates RNase L. This drug, given intravenously, has been reported to benefit CFS patients in two randomized, placebo-controlled trials.[89,90] However, the US Food and Drug Administration has repeatedly rejected a new drug application for Ampligen based on concerns about study design and efficacy. In any case, the failure of the drug to make CFS worse argues against exaggerated RNase L levels and RNA degradation as a cause of fatigue.

Role of the Central Nervous System

Because idiopathic chronic fatigue usually includes neuropsychological symptoms and subtle alterations of hormones regulated at the hypothalamic level, the hypothesis that the central nervous system (CNS) is the principal site of the pathophysiology has gained support in recent years. Accumulating data from CNS imaging studies support this notion, but the significance of these findings still is unclear.[49,91–95] Investigators have observed regions of reduced cerebral blood flow in CFS patients relative to healthy control subjects[96,95]; however, these findings were not confirmed in a study of single-photon emission computed tomography scanning in monozygotic twins discordant for CFS.[97]

Urinary free cortisol levels have been shown to be lower in patients with CFS than in age-matched and sex-matched healthy control subjects, although the means of both groups are within the defined normal range.[98,99] Exaggerated adrenal responsiveness to corticotropin infusions in these patients suggests that a subtle defect in hypothalamic-pituitary-adrenal (HPA)-axis activity exists.[100] The disturbance also results in a flattening of the diurnal pattern of cortisol secretion and enhanced sensitivity of the axis to negative feedback.[101,102] Nater and coworkers[103] reported an attenuation of morning salivary cortisol in medication-free CFS patients compared with well-matched control subjects but noted that the effect was limited to women. Reduced HPA-axis activity and hyperresponsiveness have also been observed in fibromyalgia and in posttraumatic stress disorder, whereas enhanced activity of the axis is typical of major depressive disorder.[104] In contrast, gonadotropin levels are not affected by either CFS or fibromyalgia.[105] These findings suggest the presence of a common pathophysiology in CFS and other stress-related disorders that is centered in the CNS.

The finding of depressed HPA-axis activity as a feature of chronic fatigue illnesses motivated a study at the NIH in which patients received either replacement hydrocortisone or placebo for 3 months. This study and a subsequent trial suggest that there was minor improvement in the hydrocortisone group during treatment.[106] There was also profound and sustained suppression of the HPA axis and loss of bone density, which prompted investigators to recommend against the use of steroids for treatment.[106,107] Another attempt to boost the HPA axis pharmacologically was made in a randomized controlled trial (RCT) of the drug galantamine (a central stimulant of HPA-axis activity). The study showed no substantial benefit.[108] Therefore, although HPA-axis disturbances may be present in CFS, attempts to correct these disturbances do not relieve symptoms. This suggests that any fundamental disturbance occurs at a more complex level in the brain.

The notion that the pathophysiology of CFS might involve enhanced sympathetic activity was addressed by conducting a randomized, double-blind, placebo-controlled trial of low-dose clonidine in 120 adolescent CFS patients.[109] Compared with placebo, clonidine resulted in lower levels of plasma norepinephrine and reduced C-reactive protein levels. However, the clonidine-treated patient also had fewer steps per day, suggesting that pharmacologic reduction of sympathetic outflow did not increase physical activity.

As in the earlier studies of brucellosis and influenza by Imboden and coworkers,[7] contemporary investigators have found that when patients present with a "viral illnesses" in general practice, psychiatric morbidity, belief in vulnerability to viruses, and attributional style at initial presentation are more important predictors of fatigue 6 months later than are "viral" symptoms during the initial infection.[110] Several studies have reported that a prior history of depression is frequently present in chronic fatigue states and may represent an important predisposing condition.[110–113] Perhaps the most convincing observations come from the National Child Development Study in the United Kingdom, a study that prospectively collected health and psychosocial data on huge cohorts of individuals from birth to adulthood. Cohorts born in 1946, 1958, and 1970 were analyzed in separate studies that came to similar conclusions. In the 1946 cohort 3035 participants were assessed for CFS by self-report and a structured interview at age 53 years. The study found that those with psychopathology assessed by standard interview instruments at ages 15 and 36 years were 2.65 times more likely to report CFS later in life. Moreover, the likelihood of CFS was greatest in those with the most severe psychiatric disorders.[114] This study was confirmed using the 1958 cohort of 11,419 participants.[115]

Those with psychopathology when assessed at age 33 years were 2.8 times more likely to report CFS at age 42 years. In addition, this analysis showed that psychopathology and sedentary behavior during childhood and extremes of physical activity in adulthood are not associated with CFS later in life. The association between CFS and psychopathology was highlighted by a study that found an increased suicide rate in individuals with a diagnosis of CFS.[82]

Role of the Genome

To investigate a possible genetic predisposition to chronic fatigue illnesses, family history and twin studies have been conducted. In one study CFS patients reported higher rates of fatigue illness in family members than medical control patients.[116] Similarly, twin studies in Australia and Great Britain suggest that disabling fatigue of greater than 1-month duration occurs more frequently in monozygotic than in dizygotic twins.[117,118] Applying the CDC criteria for CFS, a study in the United States showed a concordance of 38% in monozygotic twins versus 11% in dizygotic twins.[119] However, a microarray study of the transcriptomes of discordant, monozygotic twins failed to identify any biologically active marker that can distinguish the twins with CFS from those with no fatigue.[120]

With the capacity to perform genome-wide analysis, researchers now have the opportunity to search for either disease-specific alleles or to find genes that are dysregulated in CFS patients. To identify disease-specific alleles, Smith and coworkers[121] looked for polymorphisms in genes involved in the serotonergic system and found three CFS-associated alleles of the *HTR2A* gene, which encodes a G protein–coupled serotonin receptor. Polymorphisms in this gene have previously been associated with a variety of psychiatric disorders. Similarly, Rajeevan and coworkers[122] found single nucleotide polymorphisms (SNPs) in the glucocorticoid receptor *(NR3C1)* gene that occurs with relatively high frequency in CFS compared with control subjects. In a comprehensive genomic screening study Goertzel and coworkers[123] defined a panel of 28 SNPs that predicts the presence of CFS with 76% accuracy The three most relevant genes in the panel were *NR3C1, TPH2* (encoding neuronal tryptophan hydroxylase, involved in serotonin synthesis), and *COMT* (encoding catecholamine-*O*-methyl transferase). Identification of these genes suggests genetic predispositions involving the HPA axis, serotonergic system, and autonomic nervous system, respectively. These findings are consistent with functional studies that show disturbances in the targeted physiologic systems, even though the studies have identified different CFS-associated alleles.

Reconciling the differences in these genetic studies may be difficult if CFS proves to be a collection of disorders with different manifestations and predispositions. Nevertheless, research on related disorders provides strong evidence for genetic predisposition in similar functional disorders. A recent report by Binder and coworkers[124] showed that certain SNPs affecting the expression of FK506 binding protein 5 predict the development of posttraumatic stress disorder (PTSD) after adult trauma among inner-city African Americans who experienced abuse as children. Thus the combination of early psychological trauma and a genetic variant that influences the responsiveness of the glucocorticoid receptor results in hyperresponsiveness of the HPA axis in adulthood. Similarly, certain genotypes of the *MAOA* (monoamine oxidase) gene have been found to predict adult consequences in mistreated children.[125] Whether these specific genes will prove to play a role in some CFS variants is yet to be determined; however, a pathophysiologic model of genetic predisposition plus environmental challenge as the substrate for the disorder is an approach to understanding these disorders that is gaining traction.[126]

If this simple hypothesis is correct, then it should be possible to observe differences in gene expression in CFS patients versus control subjects. In accordance, a transcriptional analysis of 30,000 genes in 7 patients with postinfectious fatigue and 8 matched control subjects at four time points after documented primary EBV infection identified a panel of 35 genes with expression that correlated temporally with the clinical course.[127] Similarly, Kerr and coworkers[128] compared the transcriptomes of 25 CFS patients with those of 50 unrelated, healthy control subjects. They found 88 genes with differential expression in CFS patients. Subsequently, they identified 21 SNPs among these genes that distinguish subjects with CFS from normal and depressed subjects.[129] Unfortunately, the panels of dysregulated genes identified by these two laboratories are mutually exclusive, underscoring the heterogeneity of the syndrome, its definition, and the inherent problem of patient and control selection in CFS research.

CLINICAL MANIFESTATIONS

CFS occasionally develops in the aftermath of an identifiable infectious disease, such as infectious mononucleosis or influenza. More often, however, an infectious triggering event is not confirmed. Flulike symptoms may persist or develop de novo after the acute onset. These symptoms include sore throat, low-grade fever, tender adenopathy, generalized myalgia, migratory arthralgia, and headache. In contrast, objective physical findings corresponding to these subjective complaints, such as pharyngitis, a temperature greater than 100.5°F, and palpable adenopathy, are rare after the resolution of the initial illness. The presence of significant objective muscle weakness or frank arthritis should suggest an alternative diagnosis.

Persistent, disabling fatigue is the cardinal symptom of CFS. Exhausting fatigue that lasts hours to days may follow even modest exertion. Patients often report that they have a limited allotment of energy each day and cannot function when it is depleted. In consequence, Wessely and Powell[130] undertook a prospective study using standardized interview instruments to compare the perception of fatigue among patients with "postviral fatigue" and control groups with neuromuscular disorders or major depression. They determined that the features of physical fatigue were similar in all three groups, but complaints of mental fatigue (e.g., poor concentration, poor memory, sleepiness, decreased muscle strength), physical fatigue after mental activity, and fatigue at rest were more common in the CFS and depression groups. Mental fatigue was uncommonly reported by patients with neuromuscular disorders unless they also had a psychiatric diagnosis, and the only feature that distinguished the CFS patients from the depression patients was the tendency of the former to attribute their fatigue to a physical rather than affective cause.

Patients may describe difficulty with concentration and memory, although actual deficits are not consistently demonstrable using neuropsychological testing. Either insomnia or excessive sleep may be reported. A thorough sleep history helps to determine whether formal polysomnography is indicated to rule out a primary sleep disorder. Sleep disorders are common among patients who present with fatigue, even among patients who meet symptomatic criteria for CFS.[131–133] Any or all of the symptoms may occur persistently or occur with striking seasonality. Some cases of CFS have seasonal variation comparable with that seen with seasonal affective disorder.[134]

Most patients who meet symptom criteria for CFS also have a past or current history of a psychiatric disorder (e.g., depression or anxiety disorder).[39–40] The treating physician must determine whether a preexisting psychiatric disorder accounts for all of the patient's complaints. If so, CFS should not be diagnosed. In contrast, altered mood states that occur in the context of the CFS are often transient and reactive to the physical disability and discomfort.

It is also important for the clinician to realize that the definition of CFS intersects with several other functional somatic disorders, such as fibromyalgia (FM), irritable bowel syndrome (IBS), multiple chemical sensitivity syndrome, temporomandibular joint dysfunction, and interstitial cystitis, to name a few. Several of these disorders may occur in the same patient, and their concurrence may reflect our inability to adequately understand or define them. A recent population-based survey suggests that CFS-like illness is associated with lifetime prevalences of major depression, chronic widespread pain (i.e., FM), and IBS of 57%, 41%, and 16%, respectively.[135] Moreover, an individual with any one of these three diagnoses is 14 times more likely to have a CFS-like illness than one who has none of these three comorbid conditions. A unified view of etiology and management of these disorders is presented in a review by Henningsen and coworkers.[136]

LABORATORY FINDINGS

No laboratory tests are diagnostic for CFS. Laboratory evaluation requires testing only for the purpose of ruling out unrecognized medical

conditions that account for the symptoms. The minimal evaluation consists of a complete blood count, serum chemistry profile, urinalysis, and thyroid function testing.[21] Additional tests may be indicated when the history or quality of the patient's symptoms suggests specific alternative diseases (e.g., chronic infection, collagen vascular disease, neurologic disorder, neoplasm). A low-titer antinuclear antibody test is found in 15% to 54% of patients with CFS.[137,138] Antibodies against DNA and extractable nuclear antigens are typically absent. Instead, antibodies directed against insoluble nuclear matrix proteins are responsible for the nuclear fluorescence.[138,139] The significance of these autoantibodies is unknown.

When groups of patients with CFS are compared with control groups, significant differences have been found in many laboratory or radiologic results (e.g., in hormone levels, CNS radiologic tests, measures of cellular immunity). These findings may provide some insight into the pathophysiology of chronic fatigue states, but they have no diagnostic value in individual patients. In all of these examples there is significant overlap between the fatigued and normal groups, and the tests cannot be used as a reliable marker for the disorder in an individual. For example, although study groups of patients may have disturbances in the HPA axis, neither blood nor urine cortisol levels are sufficiently discriminatory to be of value in diagnosis of individual patients. Levels should be obtained only when clinical findings indicate a need to rule out frank adrenal insufficiency.

Orthostatic intolerance is included among the diagnostic criteria for CFS proposed by the IOM (see Table 130.3). This criterion refers to symptoms (e.g., fainting, lightheadedness, spatial disorientation, feeling dizzy or lightheaded when standing) and does not require the performance of a tilt-table test.[2] Exercise intolerance is not easily quantified. Snell and coworkers[140] observed that clinical cardiopulmonary testing did not distinguish 51 subjects with CFS from 10 sedentary but not disabled control subjects (all women) at an initial test. However, if retested 24 hours later, CFS subjects performed less well, particularly with respect to the workload at ventilatory threshold, whereas the control subjects reproduced their initial test results. Using this 2-day testing protocol, the authors correctly classified 49 of the 51 CFS cases and 9 of the 10 control subjects.

Changes in the perfusion of certain areas of the brain are suggested by studies using single-photon emission computed tomography,[91,92,95] Similarly, small areas of localized increased signal intensity, bilateral reduced white matter volume, and anisotropy of the right anterior arcuate fasciculus have been reported more frequently in brain magnetic resonance imaging studies of CFS patients than in those of healthy control subjects,[49,93] although different research groups have reported different anatomic findings. The causes of these findings are unknown, and their relationship to symptoms is not clear. Because the findings are not specific for CFS, CNS imaging should be used only when it is medically indicated to rule out a structural abnormality.

Because immunologic tests (e.g., flow cytometry, cytokine levels, in vitro lymphocyte function) have no diagnostic or prognostic significance, they are of value only in the context of a research study and should not be ordered as part of the routine evaluation for idiopathic chronic fatigue. Laboratory evaluation of the 2′,5′-oligoadenylate synthetase pathway[86] is not sufficiently specific to be helpful in diagnosis.

MANAGEMENT

General Principles

The case definitions for CFS, ME, and SEID all describe an idiopathic disorder diagnosed on the basis of clinical features, not on causation (see Tables 130.2 and 130.3). Consequently, specific medical or psychiatric therapy is rational only when an alternative or coexisting diagnosis is present. Antimicrobial therapy is not indicated unless there is clear evidence of a specific, active infection producing disease manifestations. Uncontrolled trials of successful antiviral therapy have been reported, but failed RCTs of antimicrobial therapy have been cited as evidence that conventional infectious agents are not a perpetuating factor in CFS and do not require treatment.

In my opinion, conservative therapy for idiopathic fatigue syndromes is appropriately nonspecific and should be focused on remediation of symptoms and physical rehabilitation instead of specific, presumed causes.[141] Because CFS is a heterogeneous disorder, the outcomes of treatment studies are influenced heavily by the method of selecting patients. Also, noncontrolled treatment observations are of minimal value in these disorders because most blinded controlled trials have shown a robust placebo effect.

The treatment of individual patients is empirical. It is useful to objectify the symptoms as much as possible so that the response to any intervention can be assessed independently by both the physician and the patient. Medications should be evaluated in an additive or sequential manner so that there can be no confusion about their efficacy or adverse effects. With the large range of symptoms among these patients, physicians are faced with a variety of treatments from which to choose, and patients may inquire about unconventional or alternative therapies. In the absence of evidence for efficacy, empirical treatment choices should be guided by a concern for safety and cost.

Pharmacologic Therapy

Medications may be useful for the treatment of symptoms, including nonnarcotic pain relievers for myalgia, arthralgia, or headache; nonaddictive sleep aids for sleep disruption; and psychoactive agents for depression or anxiety. Various vitamins and "nutritional supplements" have no proven, consistent benefit and may be costly.[142–146] Costly "immune-enhancing" therapies, such as intravenous immune globulin, immunostimulants, and cytokines, have failed to provide benefit in controlled studies.[147–152] A recent study examined the effect of anakinra, an antagonist of the interleukin (IL)-1α and IL-1β receptor, administered for 4 weeks in a placebo-controlled trial.[153] Patients in the study were 50 women, aged 15 to 59 years with severe fatigue attributed to CFS, who were followed for 20 weeks. No clinically significant effect on fatigue severity was found.

Carefully controlled treatment trials do not show a consistent benefit from any single pharmacologic agent. It has been useful to extrapolate from more conclusive studies of fibromyalgia patients because this idiopathic condition has significant overlap with CFS. Although the diagnosis of fibromyalgia is based on a subjective report of pain and musculoskeletal tenderness, symptoms of profound fatigue, sleep disruption, and cognitive or emotional difficulties are common accompaniments. In addition, fibromyalgia and CFS share certain biologic features (e.g., autonomic and neuroendocrine findings). Amitriptyline and serotonin-norepinephrine reuptake inhibitors (SNRIs) are moderately beneficial in fibromyalgia,[154] and common experience suggests that chronic fatigue patients with pain and sleep disorder may respond in a similar fashion.[155,156] A meta-analysis of 18 RCTs that evaluated four agents in fibromyalgia patients—milnacipran, duloxetine, pregabalin, and sodium oxybate—showed them all to have equivalent efficacy in achieving a 50% reduction of pain, with a combined mean of 31.6% of treated patients achieving this goal in all studies.[157] This mean was significantly superior to the placebo response rate, but a remarkable proportion of patients taking placebo also achieved this degree of pain reduction (18.6%). One large placebo-controlled trial of fluoxetine therapy for CFS failed to show benefit; however, this study was criticized because the treated subjects had long-standing, disabling symptoms[158] A 3-year follow-up study comparing patients who received antidepressant therapy with those who did not suggests a significant, sustained benefit of treatment; however, this analysis did not clarify the duration of therapy.[159] SNRIs, selective serotonin reuptake inhibitors, and pregabalin are available, safe, and relatively inexpensive; however, patients treated with these agents often drop out because of adverse effects or a significant nocebo (negative placebo) effect.[157] The most recent NIH workshop on therapies of CFS20 concluded that evidence was available, albeit of only fair quality, that rintatolimod, a TLR-3 agonist, may have improved some measures of exercise performance and functions of daily living.[90,160]

Nonpharmacologic Therapy

Discussions about the "reality" of CFS are decidedly unhelpful and often insulting to patients. Similarly, framing the illness in a manner that is inconsistent with the patient's perceptions (e.g., as "depression" in the absence of formal psychiatric criteria) is likely to evoke resistance and nonadherence. The best approach is the one that is most consistent

with current understanding of CFS: to recognize and validate the patient's symptoms as part of an idiopathic syndrome. In doing so, it is essential to educate the patient about the unexplained nature of the fatiguing illness and to correct any misinformation that the patient may have about its cause or treatment. This approach allows the physician to enlist the patient's support in pursuing the rational management agenda.

All patients should be counseled regarding exercise and sleep hygiene. Many patients have significant disruptions in their sleep patterns that should be corrected gradually. Daytime napping should be limited or avoided altogether because this behavior may further disrupt nighttime sleep quality. Melatonin is a popular over-the-counter sleep remedy used by many patients; however, the rationale for this therapy is unclear, given that CFS patients have normal levels and timing of endogenous melatonin secretion.[161]

Although some patients report relief of symptoms with dietary alterations, there is little reliable experimental evidence to inform changes in diet. A recent RCT showed that a low-sugar, low-yeast diet, recommended by some, was no more efficacious in relieving fatigue or improving quality of life than simple, healthy eating.[162] Highly restrictive diets that may impair general nutrition should be discouraged.

Among psychological therapies, cognitive-behavioral therapy (CBT) is considered the most effective modality in CFS patients. This approach involves a restructuring of the patient's beliefs concerning the causes of the illness and an objective assessment of the symptoms and disabilities. A 2008 meta-analysis reported successful outcome in about half of the patients entering therapy and a mean drop-out rate of 16%. The authors concluded that this modality was moderately effective.[163] The development and use of an online CBT program for Dutch adolescents likely improves the cost-effectiveness of this method as well. A randomized, open-label trial in the Netherlands showed that an Internet-based CBT program resulted in eightfold likelihood of recovery than randomization to usual care in a tertiary treatment center during a 6-month treatment period.[164] A long-term follow-up study (mean follow-up, 2.7 years) showed that the eventual recovery rate was similar in both CBT and "usual care" subjects, but the CBT-assigned subjects achieved a faster and sustained recovery after completing the online program.[165]

Individual counseling provided in general medical practice may serve the same functions and seems to be as effective as formal CBT.[166,167] However, classic insight-oriented psychotherapy rarely is indicated but may be helpful in patients who experience significant, ongoing emotional stress at home or work.

Exercise has been traditionally advocated in the treatment of fibromyalgia. Several studies have confirmed the widely held view that light aerobic exercise is also beneficial in CFS.[168–170] A systematic review of all treatment options for CFS in 2001 suggested that CBT and a program of graded exercise therapy (GET) were the only therapeutic options with credible benefit.[171] By 2011 a large randomized parallel-group trial (the *P*acing, *g*raded *A*ctivity, and *C*ognitive-behavior therapy: a randomized *E*valuation [PACE] study) was published. In this study CBT and GET both resulted in less fatigue and superior physical functioning when compared with treatment by a medical specialist or "adaptive pacing," a program in which patients control their own activity level in accordance with their perception of energy and tolerance.[172] CBT and GET also resulted in lower health care costs per quality-adjusted life year than the other nonpharmacologic therapies mentioned earlier.[173] The beneficial effects of these interventions in the PACE study were then subjected to mediation analysis that showed the most important mediator to be fear avoidance of exercise.[174] The authors concluded that abating the patient's fear of exercise should be a critical goal of CBT and GET.

Since then a 2015 NIH review of treatment similarly concluded that counseling therapies and GET were the only treatments to demonstrate benefit with at least a moderate level of evidence.[20] An updated Cochrane review of current evidence for GET reached similar conclusions.[175]

Readers of the literature and physicians who encounter patients with this syndrome should be aware that there is an active and sometimes vitriolic controversy between advocates of psychophysically oriented interventions and those who favor a standard medical (sometimes virologic) model of the disorder. The alternative hypothesis views exercise and even CBT as potentially harmful. The flavor of this controversy is apparent from the reviews of Hooper and Prins and the comments that followed[176–179] and in some of the comments after the publication of the PACE trial and the meta-analysis published by the NIH Pathways to Prevention Workshop in 2015.[180–185] Despite this opposition, there is no evidence-based benefit of bed rest as a treatment for CFS, and many physicians will likely see the potential for continuous inactivity to reinforce illness behavior and to facilitate complicating myofascial pain syndromes. In addition, there is objective evidence in adolescents and young adults with CFS of reduced range of motion of large joints and the spine compared with healthy control subjects matched for age and joint hypermobility.[186]

SUMMARY

Prolonged idiopathic fatigue states may arise spontaneously or during the convalescence from an infectious disease. When associated with other symptoms, diagnostic criteria for CFS may be met, although the distinction between syndromal and nonsyndromal chronic fatigue may be artificial. No laboratory tests are available to confirm or exclude the diagnosis of CFS. Various infections have been proposed as occult causes of the syndrome, but none has been implicated in any significant proportion of the patients who meet the clinical definition. In contrast, most experimental evidence points to a reversible derangement of certain CNS functions in persons with genetic predisposition. Treatment modalities such as exercise, CBT, and medications that act in the CNS seem to provide the most benefit.

As noted in the 2015 IOM report,[2] significant questions remain with respect to the etiology, pathophysiology, and treatment of CFS, which require rigorous and systematic research for improved understanding.

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Infectious Diseases and Their Etiologic Agents

III

A Viral Diseases

131 Biology of Viruses and Viral Diseases

James D. Chappell and Terence S. Dermody

HISTORY

Viruses have substantially influenced human health, interactions with the ecosphere, and societal history and structures. Because of their sheer numbers and ubiquity, these organisms are the single most important cause of infectious disease morbidity and mortality worldwide. The first viruses were identified as the 19th century ended. New virus identification has since continued with growing momentum. Emerging or reemerging viral diseases include bat-origin respiratory coronaviruses[1] and reoviruses[2–5]; swine-, avian-, and bat-origin influenza viruses[6,7,8]; globally spreading alphaviruses and flaviviruses that threaten the health of adults, children, and the unborn[9–11]; and highly lethal and transmissible filoviruses, such as Ebola virus.[12]

Increasing recognition of the environmental virus reservoir has been paralleled by a growing appreciation for an internal community of human viruses, collectively described as the *virome*. The human virome encompasses acute and persistent viral infections, chromosomally integrated endogenous viral elements, and bacteriophages.[13] Virome composition and dynamics carry prodigious implications for human health through direct effects on cells and tissues, interactions with innate and adaptive arms of the immune system, and influence on the bacterial microbiota. The human virome is a priority research front that will help explain virus-host interactions on the fulcrum between health and disease.

Insights into viral biology and disease have expanded in reciprocal relationship with advancements across the continuum of scientific knowledge, including the fields of microscopy, protein and nucleic acid chemistry, cell biology, immunology, and clinical medicine. In recent years, x-ray crystallography and high-resolution cryoelectron microscopy have allowed visualization of virus structures at an atomic level of resolution. Functional domains of many viral structural and enzymatic proteins have been defined, which is fostering development of new strategies to diagnose viral illnesses and design effective antiviral therapies. Viral genome detection, now represented by a multitude of routinely used target- and signal-amplification methods, has proven superior to conventional serologic assays and culture techniques for the diagnosis of many viral diseases such as those caused by enteroviruses, hepatitis viruses, herpesviruses, human immunodeficiency virus (HIV), and various respiratory and enteric viral pathogens, to name a few. Furthermore, multiplexed molecular assays ("syndromic panels") are becoming standard approaches for the simultaneous detection and differentiation of diverse viral etiologies that manifest as overlapping clinical presentations, such as acute respiratory illness and gastroenteritis. Rapid developments in nucleotide sequencing technology are permitting the application of these tools to highly sensitive and discriminatory virus detection in clinical specimens.

One of the most exciting advances in contemporary virology is the means to repurpose viruses for improving human health through systematic viral genome modification with predictable outcomes. Technologies now exist whereby specific mutations and foreign genetic material can be efficiently engineered into the genomes of most human viruses. Such approaches have been exploited in the rational design of vaccines and development of viral vectors for use in gene transduction and cancer treatment. Furthermore, these powerful techniques regularly drive research into viral pathogenesis, host responses to infection, and viral and host determinants of contagion.

Knowledge accumulation about viruses and viral diseases has been immense since the revolutionary discovery in 1882 of tobacco mosaic virus, a form of infectious agent previously unknown to the world. However, ongoing breakthrough discoveries in virology repeatedly reveal our finite comprehension of these fascinating entities, understandings of which are inseparably entwined with scientific exploration of their complex hosts.

VIRUS STRUCTURE AND CLASSIFICATION

The first classification of viruses as a group distinct from other microorganisms was based on the capacity to pass through filters of a small pore size (filterable agents). Initial subclassifications were based primarily on pathologic properties such as specific organ tropism (e.g., hepatitis viruses) or common epidemiologic features such as transmission by arthropod vectors (e.g., arboviruses). Current classification systems are based on the following: (1) the type and structure of the viral nucleic acid and the strategy used in its replication; (2) the type of symmetry of the virus capsid (helical vs. icosahedral); and (3) the presence or absence of a lipid envelope (Table 131.1).

Virus particles—virions—can be functionally defined as a delivery system that surrounds a payload. The delivery system consists of structural components used by the virus to survive in the environment and bind

TABLE 131.1 Classification of Viruses

FAMILY	EXAMPLE	TYPE OF NUCLEIC ACID	GENOME SIZE (kb OR kb PAIR)	ENVELOPE	CAPSID SYMMETRY
RNA-Containing Viruses					
Picornaviridae	Poliovirus	SS (+) RNA	7–9	No	I
Astroviridae	Astrovirus	SS (+) RNA	6–8	No	I
Caliciviridae	Norwalk virus	SS (+) RNA	7–8	No	I
Togaviridae	Rubella virus	SS (+) RNA	10–12	Yes	I
Flaviviridae	Yellow fever virus	SS (+) RNA	9–13	Yes	S
Coronaviridae	Coronavirus	SS (+) RNA	26–32	Yes	H
Rhabdoviridae	Rabies virus	SS (–) RNA	11–15	Yes	H
Paramyxoviridae	Measles virus	SS (–) RNA	13–18	Yes	H
Filoviridae	Ebola virus	SS (–) RNA	19	Yes	H
Arenaviridae	Lymphocytic choriomeningitis virus	2 SS (ambisense) RNA segments	11	Yes	S
Bunyaviridae	California encephalitis virus	3 SS (ambisense) RNA segments	11–19	Yes	H
Orthomyxoviridae	Influenza virus	6–8 SS (–) RNA segments	10–15	Yes	H
Reoviridae	Rotavirus	10–12 DS RNA segments[a]	19–32	No	I
Retroviridae	Human immunodeficiency virus	2 identical SS (+) RNA segments	7–13	Yes	S
DNA-Containing Viruses					
Hepadnaviridae	Hepatitis B virus	Circular DS DNA with SS portions	3–4	Yes	I
Parvoviridae	Human parvovirus B19	SS (+) or (–) DNA	4–6	No	I
Polyomaviridae	JC virus	Circular DS DNA	5	No	I
Papillomaviridae	Human papillomavirus	Circular DS DNA	7–8	No	I
Adenoviridae	Adenovirus	Linear DS DNA	26–48	No	I
Herpesviridae	Herpes simplex virus	Linear DS DNA	125–240	Yes	I
Poxviridae	Vaccinia virus	Linear DS DNA	130–375	Yes	Complex

[a]Reovirus and orbivirus, 10 segments; rotavirus, 11 segments; Colorado tick fever virus, 12 segments.
(+), Message sense; (–), complement of message sense; *DS,* double-stranded; *H,* helical; *I,* icosahedral; *S,* spherical; *SS,* single-stranded.
Data from Condit RC. Principles of virology. In: Knipe DM, Howley PM, eds. Fields Virology. *6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013:21–51.*

to host cells. The payload contains the viral genome and often includes enzymes required for the initial steps in viral replication. In almost all cases, the delivery system must be removed from the virion to allow viral replication to commence.

In addition to mediating attachment to host cells, the delivery system also plays a crucial role in determining the mode of transmission between hosts. Viruses containing lipid envelopes are sensitive to desiccation in the environment and, for the most part, are transmitted by the respiratory, parenteral, and sexual routes. Nonenveloped viruses are stable to harsh environmental conditions and are often transmitted by the fecal-oral route.

Viral genomes exist in a variety of forms and sizes and consist of DNA or RNA (see Table 131.1). Animal virus genomes range in size from 3 kb, encoding only three or four proteins in small viruses such as the hepadnaviruses, to more than 300 kb, encoding several hundred proteins in large viruses such as the poxviruses. Viral genomes are single- or double-stranded and circular or linear. RNA genomes are composed of a single molecule of nucleic acid or multiple discrete segments, which can vary in number from as few as two in the arenaviruses up to 12 in some members of the Reoviridae. Viral nucleic acid is packaged in a protein coat, or capsid, that consists of multiple protein subunits. The combination of the viral nucleic acid and the surrounding protein capsid is often referred to as the *nucleocapsid* (Fig. 131.1).

Structural details of many viruses have now been defined at an atomic level of resolution (Fig. 131.2). General features of virus structure can be gained from examination of electron micrographs of negatively stained virions and thin-section electron micrographs of virus-infected tissues and cultured cells. These techniques allow rapid identification of viral size, shape, symmetry, and surface features; presence or absence of an envelope; and intracellular site of viral assembly. Cryoelectron microscopy and computer image processing techniques are used to determine the three-dimensional structures of spherical viruses at a level of resolution far superior to that of negatively stained electron micrographs. A major advantage of cryoelectron microscopy is that it allows studies of viruses to be performed under conditions that do not alter native virion structure. Moreover, advances in cryoelectron microscopy have extended the achievable resolution of particle-associated proteins to atomic levels, sufficient to recognize characteristic features of secondary structural elements.[14] Image reconstructions of cryoelectron micrographs, sometimes in combination with x-ray crystallography, also can be used to investigate structural aspects of various virus functions, including receptor binding[15–17] and interaction with antibodies.[18,19] Identification of key motifs, such as receptor-binding sites or immunodominant domains, provides the framework for understanding the structural basis of virus-cell interactions. Electron tomography with image reconstruction has been applied to architectural studies of viruses and intracellular foci of virus replication, rendering exquisite three-dimensional representations of particle organization and revealing the structure and subcellular origins of virus assembly centers.[20,21]

A number of general principles have emerged from studies of virus structure. In almost all cases, the capsid is composed of a repeating series of structurally similar subunits, each of which in turn is composed of only a few different proteins. The parsimonious use of structural proteins in a repetitive motif minimizes the amount of genetic information required to encode the viral capsid and leads to structural arrangements with symmetrical features. All but the most complex viruses exhibit either helical or icosahedral symmetry (see Table 131.1). Viruses with helical symmetry contain repeating protein subunits bound at regular intervals along a spiral formed by the viral nucleic acid. Interesting

to note, all known animal viruses that show this type of symmetry have RNA genomes. Viruses with icosahedral symmetry display twofold, threefold, and fivefold axes of rotational symmetry, and viral nucleic acid is intimately associated with specific capsid proteins in an ordered packing arrangement.

The use of repeating subunits with symmetrical protein-protein interactions facilitates the assembly of the viral capsid. In most cases, viral assembly appears to be a spontaneous process that occurs under the appropriate physiologic conditions and often can be reproduced when recombinant viral proteins are expressed in the absence of viral replication.[22,23] For many viruses, assembly of the capsid proceeds through a series of intermediates, each of which nucleates the addition of subsequent components in the assembly sequence.

One of the least understood aspects of viral assembly is the process that ensures that the viral nucleic acid is correctly packaged into the capsid. In the case of viruses with helical symmetry, there may be an initiation site on the nucleic acid to which the initial capsid protein subunit binds, triggering the addition of subsequent subunits. The genomes of most DNA-containing viruses are inserted into preassembled capsid intermediates (procapsids) through adenosine triphosphate–driven mechanisms.[24] In preparations of many icosahedral viruses, empty capsids (i.e., capsids lacking nucleic acid) are frequently observed, indicating that assembly may proceed to completion without a requirement for the viral genome.

In some viruses, the nucleocapsid is surrounded by a lipid envelope acquired as the virus particle buds from the host cell cytoplasmic, nuclear, or endoplasmic reticular membrane (see Fig. 131.1). Inserted into this lipid bilayer are virus-encoded proteins (e.g., the hemagglutinin [HA] and neuraminidase proteins of influenza virus and gp41 and gp120 of HIV), which are exposed on the surface of the virus particle. These viral proteins usually contain a glycosylated hydrophilic external portion and internal hydrophobic domains that span the lipid membrane and anchor the protein into the viral envelope. In some cases, another viral protein, often termed a *matrix protein,* associates with the internal (cytoplasmic) surface of the lipid envelope, where it can interact with the cytoplasmic domains of the envelope glycoproteins. Matrix proteins may function in stabilizing the interaction between viral glycoproteins and the lipid envelope, directing the viral genome to intracellular sites of viral assembly or viral budding. Matrix proteins also can influence a diverse set of cellular functions to create a permissive replication environment, such as inhibition of host cell transcription[25,26] and evasion of the cellular innate antiviral response.[27]

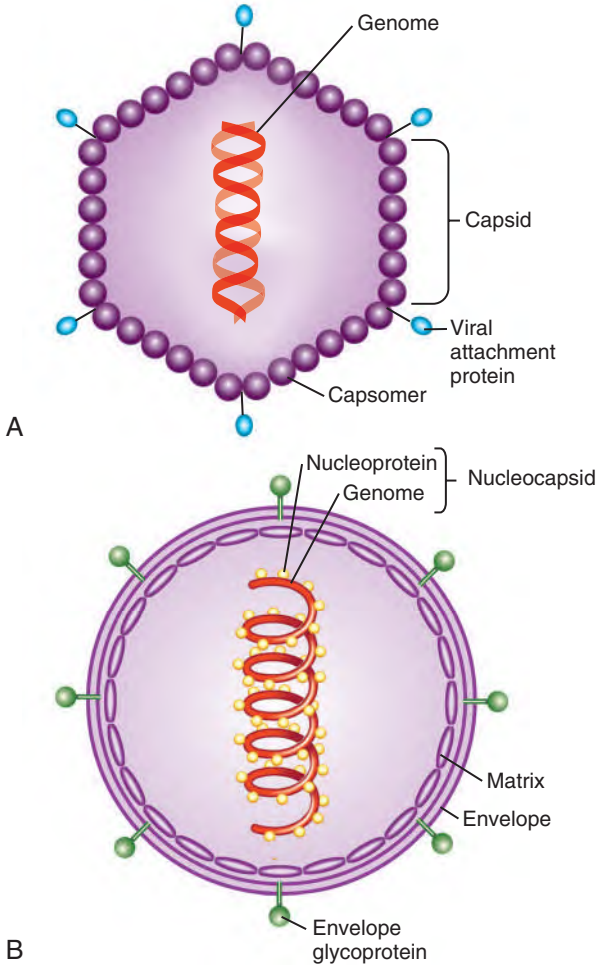

FIG. 131.1 Schematic diagrams illustrating the structure of a nonenveloped icosahedral virus (A) and an enveloped helical virus (B). Nucleocapsid: combination of a viral nucleic acid and surrounding protein capsid.

VIRUS-CELL INTERACTIONS

Viruses require an intact cell to replicate and can direct the synthesis of hundreds to thousands of progeny viruses during a single cycle of infection. In contrast to other microorganisms, viruses do not replicate by binary fission. Instead, the infecting particle must disassemble in order to multiply itself.

Attachment

The interaction between a virus and its host cell begins with attachment of the virus particle to specific receptors on the cell surface. Viral proteins that mediate the attachment function (viral attachment proteins) include the following: single-capsid components that extend from the virion surface, such as the attachment proteins of adenovirus,[28] reovirus,[29] and

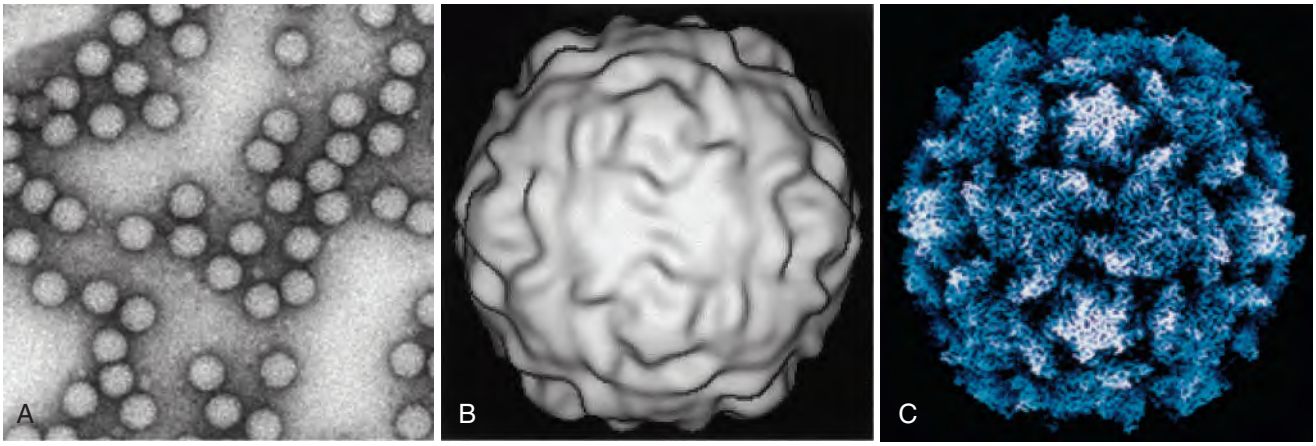

FIG. 131.2 Structural studies of poliovirus. (A) Negative-stained electron micrograph. (B) Three-dimensional image reconstruction of cryoelectron micrographs. (C) Structure determined by x-ray crystallography. *(Courtesy Dr. James Hogle, Harvard University.)*

rotavirus[30,31]; surface glycoproteins of enveloped viruses, such as influenza virus[32,33] (Fig. 131.3) and HIV[34,35]; viral capsid proteins that form binding pockets that engage cellular receptors, such as the canyon formed by the capsid proteins of poliovirus[36] and rhinovirus[37]; and viral capsid proteins that contain extended loops capable of binding receptors, such as foot-and-mouth disease virus.[38] Studies of the attachment of several diverse virus groups, including adenoviruses, coronaviruses, herpesviruses, lentiviruses, and reoviruses, indicate that multiple interactions between virus and cell occur during the attachment step. These observations indicate that a particular sequence of binding events between virus and cell optimizes specificity and contributes significant stability to the association.[39]

One of the most dynamic areas of virology concerns the identification of virus receptors on host cells. This interest stems in part from the critical importance of the attachment step as a determinant of host species tropism and target cell selection by many viruses. Numerous virus receptors have been identified (Table 131.2), and three important principles have emerged from studies of these receptors. First, viruses have adapted to use cell surface molecules designed to facilitate a variety of normal cellular functions. Virus receptors may be highly specialized proteins with limited tissue distribution, such as complement receptors, growth factor receptors, or neurotransmitter receptors, or more ubiquitous components of cellular membranes, such as integrins and other intercellular adhesion molecules, glycosaminoglycans, or sialic acid–containing oligosaccharides. Second, many viruses use more than a single receptor to mediate multistep attachment and internalization. For example, adenovirus binds coxsackievirus-adenovirus receptor (CAR)[40] and the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$[41]; herpes simplex virus (HSV) binds heparan sulfate[42–44] and herpesvirus entry mediator (HVEM/HveA),[45] nectin 1 (PRR1/HveC),[46] or nectin 2 (PRR2/HveB)[47]; HIV binds CD4[48,49] and chemokine receptors CXCR4[50,51] and CCR5[52–54]; and reovirus binds sialylated glycans,[55,56] junctional adhesion molecule A (JAM-A),[57,58] and NgR1.[59] Third, in many cases, receptor expression is not the sole

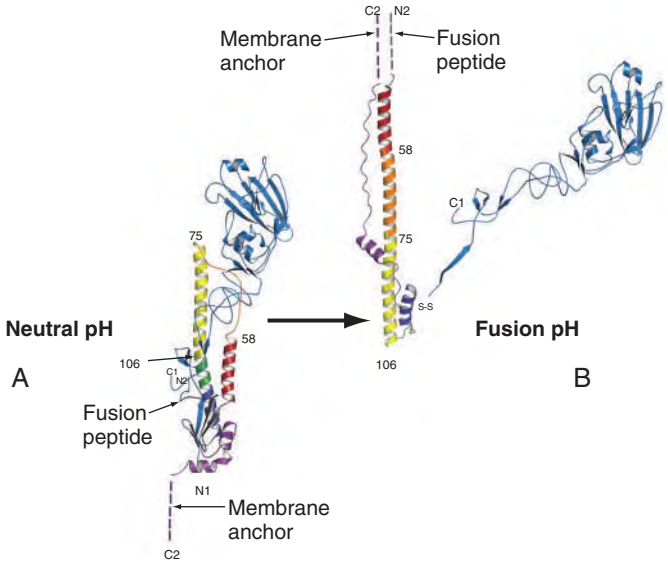

FIG. 131.3 The folded structure of the influenza virus hemagglutinin (HA) and its rearrangement when exposed to low pH. (A) The HA monomer. HA1 is blue, and HA2 is multicolored. The receptor-binding pocket resides in the virion-distal portion of HA1. The viral membrane would be at the bottom of this figure. (B) Conformational change in HA induced by exposure to low pH. Note the dramatic structural rearrangement in HA2, in which amino-acid residues 40 to 105 become a continuous alpha helix. *Dashed lines* indicate regions of undetermined structure. This model of HA in its fusion conformation is a composite of the HA1 domain structure and the low-pH HA2 structure. *(Modified from Russell RJ, Kerry PS, Stevens DJ, et al. Structure of influenza hemagglutinin in complex with an inhibitor of membrane fusion.* Proc Natl Acad Sci U S A. *2008;105: 17736–17741.)*

TABLE 131.2 Receptors and Entry Mediators Used by Selected Human Viruses

VIRUS	RECEPTOR
Adeno-associated virus	AAVR[291]
Adenovirus	Coxsackievirus and adenovirus receptor (CAR)[40,286] CD46[287,288] Desmoglein 2[289] Integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$[41] Sialic acid–containing oligosaccharides[290]
Coronavirus	9-*O*-acetylated sialic acid–containing oligosaccharides (HCoV-OC43[292] and HCoV-HKU-1[293]) Aminopeptidase N (HCoV-229E)[294,295] Angiotensin-converting enzyme 2 (SARS-CoV[296] and NL63[297]) Dipeptidyl peptidase 4 (MERS-CoV)[298] Carcinoembryonic antigen–related cell adhesion molecule 5 (MERS-CoV)[299] α2,3-linked sialic acids (MERS-CoV)[300]
Coxsackievirus	Integrin $\alpha_v\beta_3$[301] Decay-accelerating factor (CD55)[302,303] Coxsackievirus and adenovirus receptor (CAR)[40] Intercellular adhesion molecule 1 (ICAM-1)[304] GRP78/BiP[305] Heparan sulfate[306] Sialic acid[307]
Cytomegalovirus	Heparan sulfate[308,309] Integrins $\alpha_2\beta_1$, $\alpha_6\beta_1$, $\alpha_v\beta_3$[310] Platelet-derived growth factor-α receptor[311]
Ebola virus	T-cell immunoglobulin and mucin domain 1 (TIM-1)[315] Niemann-Pick C1 cholesterol transporter[316,317]
Echovirus	Integrin $\alpha_2\beta_1$[312] Decay accelerating factor (CD55)[313,314]
Enterovirus 68	ICAM-5 (telencephalin)[318]
Enterovirus 71	P-selectin glycoprotein ligand-1 (PSGL-1)[319] Scavenger receptor B2 (SR-B2)[320]
Epstein-Barr virus	Complement receptor 2 (CD21)[321,322] MHC class II protein[75]

TABLE 131.2 Receptors and Entry Mediators Used by Selected Human Viruses—cont'd

VIRUS	RECEPTOR
Hantaviruses	β_3 Integrins[323]
Henipaviruses	Ephrin-B2[324,325] Ephrin-B3[326]
Hepatitis A virus	Mucin-like protein TIM-1[327]
Hepatitis C virus	CD81[328,329] Scavenger receptor B1 (SRB1)[330,331] Claudin[332] Occludin[333]
Herpes simplex virus	Heparan sulfate[42–44] Herpesvirus entry mediator (HVEM/HveA)[45] Nectin 1 (PRR1/HveC)[46] Nectin 2 (PRR2/HveB)[47]
Human herpesvirus 6A	CD46[334]
Human herpesvirus 6B	CD134[335]
Human immunodeficiency virus	CD4[48,49] Chemokine receptor CXCR4[50,51] Chemokine receptor CCR5[52–54]
Human metapneumovirus	Integrin $\alpha_v\beta_1$[336]
Human T-cell leukemia virus	Glucose transporter GLUT-1[337] Neuropilin-1[338]
Influenza virus	Sialic acid–containing oligosaccharides[33,339]
Kaposi sarcoma herpesvirus	Heparan sulfate[340] Integrin $\alpha_3\beta_1$[341] Ephrin-A2[342]
Measles virus	CD46 (vaccine strains)[343,344] Signaling lymphocyte-activation molecule (SLAM)[345] Nectin-4[346,347]
Mumps virus	Sialic acid[348]
New World hemorrhagic fever arenaviruses (e.g., Junin virus)	Transferrin receptor 1[349]
Norovirus	Histo-blood group antigens[350,351] Gangliosides[352]
Old World hemorrhagic fever arenaviruses (e.g., Lassa fever virus)	α-Dystroglycan[353] Lysosome-associated membrane protein-1[354]
Parvovirus B19	Erythrocyte P antigen (globoside)[355]
Poliovirus	Poliovirus receptor (PVR, CD155)[191]
Polyomavirus (BK)	Gangliosides GD1b and GT1b[356,357]
Polyomavirus (JC)	Serotonin receptor 5HT2A[358] LSTc pentasaccharide[72]
Polyomavirus (Merkel cell)	Heparan sulfate[359] Sialic acid[360,361]
Rabies virus	Neural cell adhesion molecule (CD56)[362] Nerve growth factor receptor (P75NTR)[363]
Reovirus	Sialic acid–containing oligosaccharides[55,56] Junctional adhesion molecule-A (JAM-A)[57] Nogo receptor-1 (NgR1)[364] β_1 integrins[365]
Rhinovirus (type A, major group, and type B)	Intercellular adhesion molecule 1 (ICAM-1)[366–368]
Rhinovirus (type A, minor group)	Low-density lipoprotein receptor[369]
Rhinovirus (type C)	Cadherin-related family member 3[370]
Rotavirus	Sialic acid–containing oligosaccharides[371,372] Histo-blood group antigens[373] Integrins $\alpha_2\beta_1$, $\alpha_4\beta_1$, $\alpha_v\beta_3$, $\alpha_x\beta_2$[374,375]
Rubella virus	Myelin oligodendrocyte glycoprotein (MOG)[376]
Sindbis virus	Natural resistance–associated macrophage protein (NRAMP)[377]

determinant of viral tropism for particular cells and tissues in the host. Therefore, although receptor binding is the first step in the interaction between virus and cell, subsequent events in the viral replication cycle also must be supported for productive viral infection to occur.

Several viruses bind receptors expressed at regions of cell-cell contact.[60] JAM-A, which serves as a receptor for reovirus[57] and feline calicivirus,[61] and CAR, which serves as a receptor for some coxsackieviruses and adenoviruses,[40] are expressed at tight junctions[62,63] and adherens junctions.[64,65] Junctional regions are sites of enhanced membrane recycling, endocytic uptake, and intracellular signaling.[66] Therefore it is possible that viruses have selected junction-associated proteins as receptors to usurp the physiologic functions of these molecules. In this regard, interactions of coxsackievirus with decay-accelerating factor elicit a tyrosine kinase–based signaling cascade that mediates subsequent interactions of the virus with CAR in tight junctions.[67] Structures of viral proteins or whole viral particles in complex with sialic acid have been determined for some viruses, including the influenza virus HA[33,68] (see Fig. 131.3), polyomavirus,[69–72] foot-and-mouth disease virus,[73] reovirus attachment protein σ1,[55,56] and the VP8 domain of rotavirus capsid protein VP4.[31] Sialic acid binding in each of these cases occurs in a shallow groove at the surface of the viral protein. However, the architectures of the binding sites differ. Structures of complexes of viral proteins or viral particles and cell surface protein receptors have also been determined. These include adenovirus fiber knob and CAR,[74] Epstein-Barr virus (EBV) gp42 and major histocompatibility complex (MHC) class II protein,[75] HSV glycoprotein D and HVEM/HveA,[76] HIV gp120 and CD4,[35] measles virus HA and CD46[77] and SLAM (signaling lymphocyte-activation molecule),[78] reovirus σ1 and JAM-A,[58] and rhinovirus and ICAM-1 (intercellular adhesion molecule 1).[79] In several of these cases, the viral attachment proteins engage precisely the same domains used by their cognate receptors to bind natural ligands.

Penetration and Disassembly

Once attachment has occurred, the virus must penetrate the cell membrane, and the capsid must undergo a series of disassembly steps (uncoating) that prepare the virus for the next phases in viral replication. Enveloped viruses including some paramyxoviruses and retroviruses enter cells by fusion of the viral envelope with the cell membrane (Fig. 131.4). Attachment of these viruses to the cell surface induces changes in viral envelope proteins required for membrane fusion. For example, the binding of CD4 and certain chemokine receptors by HIV envelope glycoprotein gp120 induces a series of conformational changes in gp120 that lead to the exposure of transmembrane protein gp41.[80,81] Fusion of viral and cellular membranes proceeds through subsequent interactions of the hydrophobic gp41 fusion peptide with the cell membrane.[82,83,84,85]

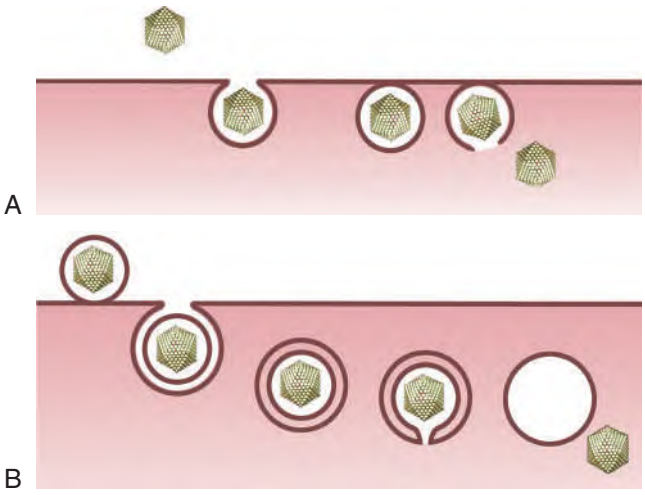

FIG. 131.4 Mechanisms of viral entry into cells. Nonenveloped (A) and enveloped (B) virus internalization by receptor-mediated endocytosis.

Other viruses enter cells by some form of receptor-mediated endocytic uptake (see Fig. 131.4). For several viruses, virus-receptor complexes induce formation of clathrin-coated pits that invaginate from the cell membrane to form coated vesicles.[86] These vesicles are rapidly uncoated and fuse with early endosomes, which sort internalized proteins for recycling to the cell surface or other cellular compartments, such as late endosomes or lysosomes. For other viruses, virus-receptor complexes are taken into cells by caveolae in lipid rafts.[86] Enveloped viruses such as dengue virus,[87] influenza virus,[88] and Semliki Forest virus[89] exploit the acidic environment of the endocytic compartment to induce conformational changes in surface glycoproteins required for membrane fusion. High-resolution structures of the influenza virus HA at acidic pH illustrate a dramatic conformational alteration leading to the fusion-active state (see Fig. 131.3).[88]

Endocytic uptake and acidification are also required for entry of some nonenveloped viruses such as adenovirus,[90,91] parvovirus,[92] and reovirus.[93,94] In these cases, acidic pH may facilitate disassembly of the viral capsid to enable subsequent penetration of endosomal membranes. In addition to acidic pH, endocytic cathepsin proteases are required for disassembly of several viruses, including Ebola virus,[95] Hendra virus,[96] reovirus,[97] and Middle East respiratory syndrome[98] and severe acute respiratory syndrome (SARS)[99] coronaviruses.

In contrast to enveloped viruses, nonenveloped viruses cross cell membranes using mechanisms that do not involve membrane fusion. This group of viruses includes several human pathogens, with adenoviruses, picornaviruses, and rotaviruses serving as prominent examples. Despite differences in genome and capsid composition, each of these viruses must penetrate cell membranes to deliver the genetic payload to the interior of the cell. Capsid rearrangements triggered by receptor binding,[100,101] acidic pH,[90,91] or proteolysis[102,103] serve essential functions in membrane penetration by some nonenveloped viruses. Although a precise understanding of the biochemical mechanisms that underlie viral membrane penetration is incomplete, small capsid proteins of several nonenveloped viruses, such as adenovirus,[104] poliovirus,[105] and reovirus,[106] are required for membrane penetration, perhaps by forming pores in host cell membranes.

Genome Replication

Once a virus has entered a target cell, it must replicate its genome and synthesize its proteins. Replication strategies used by single-stranded, RNA-containing viruses depend on whether the genome can be used as messenger (m)RNA. Translation-competent genomes, which include those of the coronaviruses, flaviviruses, picornaviruses, and togaviruses, are termed *plus* (+) *sense* and are translated by cellular ribosomes immediately following entry of the genome into the cytoplasm. For most viruses containing (+) sense RNA genomes, translation results in synthesis of a large polyprotein that is cleaved into several smaller proteins through the action of viral and sometimes host proteases. One of these proteins is an RNA-dependent RNA polymerase (RdRp), which replicates the viral RNA. Genome replication of (+) sense RNA-containing viruses requires synthesis of a minus (−) sense RNA intermediate, which serves as template for production of (+) sense genomic RNA.

A different strategy is used by viruses containing (−) sense RNA genomes. The genomes of these viruses, which include the filoviruses, orthomyxoviruses, paramyxoviruses, and rhabdoviruses, cannot serve directly as mRNA. Therefore viral particles must contain a copackaged RdRp to transcribe (+) sense mRNAs using the (−) sense genomic RNA as template. Genome replication of (−) sense RNA-containing viruses requires synthesis of a (+) sense RNA intermediate, which serves as a template for production of (−) sense genomic RNA. Mechanisms that determine whether (+) sense RNAs are used as templates for translation or genome replication are not well understood.

RNA-containing viruses belonging to the family Reoviridae have segmented, double-stranded (ds) RNA genomes. The innermost protein shell of these viruses (termed a *single-shelled particle* or *core*) contains an RdRp that catalyzes the synthesis of (+) sense mRNA using as a template the (−) sense strand of each dsRNA segment. The mRNAs of these viruses are capped at their 5′-termini by virus-encoded enzymes and then extruded into the cytoplasm through channels in the single-shelled particle.[107] The (+) sense mRNAs also serve as a template for

replication of dsRNA gene segments. Viral genome replication is thus completely conservative; neither strand of parental dsRNA is present in newly formed genomic segments.

The retroviruses are RNA-containing viruses that replicate using a DNA intermediate. The viral genomic RNA is (+) sense and single stranded; however, it does not serve as mRNA following viral entry. Instead, the retrovirus RNA genome is a template for synthesis of a double-stranded DNA copy, termed the *provirus.* Synthesis of the provirus is mediated by a virus-encoded RNA-dependent DNA polymerase or *reverse transcriptase,* so named because of the reversal of genetic information from RNA to DNA. The provirus translocates to the nucleus and integrates into host DNA. Expression of this integrated DNA is regulated for the most part by cellular transcriptional machinery. However, the human retroviruses HIV and human T-cell leukemia virus (HTLV) encode proteins that augment transcription of viral genes. Intracellular signaling pathways are capable of activating retroviral gene expression and function, inducing high levels of viral replication in response to certain stimuli.[108] Transcription of the provirus yields mRNAs that encode viral proteins and genome-length RNAs that are packaged into progeny virions. Such a replication strategy results in persistent infection in the host because the viral genome is maintained in the host cell genome and replicated with each cell division.

With the exception of the poxviruses, viruses containing DNA genomes replicate in the nucleus and for the most part use cellular enzymes for transcription and replication of their genomes. Transcription of most DNA-containing viruses is tightly regulated and results in the synthesis of early and late mRNA transcripts. The early transcripts encode regulatory proteins and proteins required for DNA replication, whereas the late transcripts encode mainly structural proteins. Several DNA-containing viruses, such as adenovirus and human papillomavirus (HPV), induce cells to express host proteins required for viral DNA replication by stimulating cell-cycle progression. For example, the HPV E7 protein binds the retinoblastoma gene product pRB and liberates transcription factor E2F, which induces the cell cycle.[109,110] To prevent programmed cell death in response to E7-mediated unscheduled cell-cycle progression, the HPV E6 protein mediates the ubiquitylation and degradation of tumor suppressor protein p53.[111–113]

Some DNA-containing viruses, such as the herpesviruses, can establish latent infections in the host. Unlike the retroviruses, genomes of the herpesviruses do not integrate into host chromosomes but instead exist as plasmid-like episomes. Mechanisms that govern establishment of latency and subsequent reactivation of replication are not well understood. However, microRNAs encoded by cytomegalovirus (CMV) and perhaps other herpesviruses may promote persistence by targeting viral and cellular mRNAs that control viral gene expression and replication and innate immune responses to viral infection.[114,115]

A fascinating aspect of virus-cell interactions is the replication microenvironments established in infected cells. Viral replication is a sophisticated interplay of transcription, translation, nucleic acid amplification, and particle assembly. Furthermore, infection must proceed under sensitive pathogen surveillance systems trained on virus-associated molecular patterns (e.g., unmethylated CpG dinucleotides in viral DNA genomes and exposed 5′ triphosphates of many viral RNAs) and replicative intermediates (e.g., dsRNA generated during RNA virus replication) that may impose impassable blocks to infection.[116] Partitioning of the viral replication machinery from the surrounding intracellular milieu satisfies a spatial requirement to concentrate viral proteins and nucleic acid for efficient genome amplification and encapsidation while simultaneously shielding viral products from cellular sensors that provoke antiviral innate immune responses. Hence, as a general rule, viral replication is a localized process, occurring within morphologically discrete cytoplasmic or nuclear structures variously termed *viral inclusions* (or *inclusion bodies*), *virosomes, viral factories,* or *viroplasms.* These entities are novel, metabolically active organelles formed by contributions from both virus and cell. Many highly recognizable features of viral cytopathic effect observed using light microscopy, such as dense nuclear inclusions or refractile cytoplasmic densities, represent locally concentrated regions of viral nucleic acid and protein.

Membrane-associated replicase complexes appropriated by (+) sense RNA viruses are perhaps the most conspicuous examples of compartmentalized viral replication. In cells infected by these viruses, intracellular membranes originating from the endoplasmic reticulum (ER; e.g., picornaviruses[117,118]), ER-Golgi intermediate compartment and *trans*-Golgi network (e.g., flaviviruses[119]), endolysosomal vesicles (e.g., alphaviruses[120]), and autophagic vacuoles (e.g., poliovirus[121]) are reduplicated and reorganized by viral proteins into platforms that anchor viral replication complexes consisting of the RdRp and other RNA-modifying enzymes required for RNA synthesis. Viruses with dsRNA genomes also form replication organelles composed of host-derived membranes,[122] the nature of which is less clear. The assembly pathway of rotavirus, a dsRNA virus, involves budding of immature particles into the ER, where a lipid envelope is transiently acquired and subsequently replaced by the outermost protein shell.[123]

The tight relationship of RNA virus replication to cellular membranes is less predictable for DNA viruses. For example, in distinction to the supporting role of autophagy in the replication of some RNA viruses, autophagosomes (stress-induced, double-membraned vesicles that remove noxious cytoplasmic materials to lysosomes for degradation) defend against infection by HSV-1, which encodes a protein that inhibits induction of autophagy and accentuates viral virulence.[124,125] The replication and assembly complexes of many DNA viruses, including adenoviruses, herpesviruses, papillomaviruses, polyomaviruses, and parvoviruses, are associated with promyelocytic leukemia (PML) nuclear bodies,[126,127] which have been ascribed functions in diverse nuclear processes encompassing gene regulation, tumor suppression, apoptosis, and removal of aggregated or foreign proteins.[128] It appears that DNA viruses exploit PML bodies in a variety of ways, which include consolidation and disposal of misfolded viral proteins, sequestration of host cell stress-response factors that block infection, and segregation of interfering cellular DNA repair proteins from sites of viral replication.[129]

The life cycles of all viruses that replicate in eukaryotic cells are physically and functionally intertwined with the cytoskeleton. Many viruses with nuclear replication programs, such as adenovirus, HSV, and influenza virus, are transported by motor proteins along microtubules toward the nucleus, resulting ultimately in release of the viral genome into the nucleoplasm through nuclear pores.[130] The microtubule network is also conscripted as an egress pathway by a number of enveloped viruses (e.g., HIV, HSV, and vaccinia virus) for conveyance of immature particles to sites of virion budding.[131] Furthermore, microtubules and actin filaments may serve as anchorage points for nucleoprotein complexes that coordinate genome expression or amplification with cytoplasmic replication programs, exemplified by parainfluenza virus (PIV),[132] reovirus,[133] and vaccinia virus.[134] Because the cytoskeleton is a decentralized organelle linking cellular structural elements to the metabolic and transport machineries, it is not surprising that viruses capitalize on this highly integrative system, which provides a stable platform for replication and enables purposeful movement of virions or subviral components within cells to facilitate the requisite insulation of viral assembly from disassembly.

Release

Exit of progeny virions from an infected cell classically has been understood to occur via budding by enveloped viruses (directly from the cell membrane as in the case of HIV[135] or into a secretory pathway as exemplified by hepatitis C virus [HCV][136]) or cell lysis by nonenveloped viruses. In addition, outgoing particles have been considered to be typically disconnected infectious units, independent of one another. These concepts of virus release likely are oversimplified because emerging evidence supports an alternative mode of viral egress involving extracellular vesicles.[137] A variety of nonenveloped and enveloped viruses appear to migrate through the extracellular milieu as micropopulations within cell-derived vesicles originating from multivesicular bodies or autophagosomes. This transit strategy confers many possible advantages to virus survival—for example, invisibility to immune surveillance systems and genetic robustness allowing for rapid adaptation to different replication environments. The group paradigm of a viral infectious unit carries numerous implications for theoretical and experimental virology, particularly in the areas of viral evolution, quasispecies dynamics, clonality, population diversity within an infectious focus, and cell attachment and entry mechanisms.

Cell Killing

Viral infection can compromise numerous cellular processes, such as nucleic acid and protein synthesis, maintenance of cytoskeletal architecture, and preservation of membrane integrity.[138] Many viruses are also capable of inducing genetically programmed mechanisms of cell death including apoptosis and pyroptosis.[139,140,141] Apoptotic cell death is characterized by cell shrinkage, membrane blebbing, condensation of nuclear chromatin, and activation of an endogenous endonuclease, which cleaves cellular DNA into oligonucleosome-length DNA fragments.[142] These changes occur according to predetermined developmental programs or in response to certain environmental stimuli. In some cases, apoptosis may serve as an antiviral defense mechanism to limit viral replication by destruction of virus-infected cells or reduction of potentially harmful inflammatory responses elicited by viral infection.[143] In other cases, apoptosis may result from viral induction of cellular factors required for efficient viral replication.[139,140] In general, RNA-containing viruses, including influenza virus, measles virus, poliovirus, reovirus, and Sindbis virus, induce apoptosis of host cells, whereas DNA-containing viruses, including adenovirus, CMV, EBV, HPV, and the poxviruses, encode proteins that block apoptosis. For some viruses, the duration of the viral infectious cycle may determine whether apoptosis is induced or inhibited. Viruses capable of completing an infectious cycle before induction of apoptosis would not require a means to inhibit this cellular response to viral infection. Interesting to note, several viruses that cause encephalitis are capable of inducing apoptosis of infected neurons (Fig. 131.5).[144–146]

Pyroptosis also has been recognized as a form of programmed cell death in response to intracellular pathogens.[147] This system is stimulated by cellular sensors *(inflammasomes)* of microbial components, triggering activation of caspases and subsequent cleavage of gasdermin D and precursor forms of the proinflammatory mediators interleukin (IL)-1β and IL-18 into biologically active products. Membrane pore formation mediated by a gasdermin D–derived polypeptide leads to release of mature IL-1β and IL-18 and cytolemmal rupture. The precise roles of pyroptosis in viral replication and pathogenesis await results of ongoing studies. CD4 T-cell decline during HIV infection has been linked to pyroptosis.[148]

ANTIVIRAL DRUGS

(Also see Chapters 44 to 48.)

Knowledge of viral replication strategies has provided insights into critical steps in the viral life cycle that can serve as potential targets for antiviral therapy. For example, drugs can be designed to interfere with virus binding to target cells or prevent penetration and disassembly once receptor engagement has occurred. Steps involved in replication of the viral genome are also obvious targets for antiviral therapy. A number of antiviral agents inhibit viral polymerases, including those active against herpesviruses (e.g., acyclovir), HIV (e.g., zidovudine), hepatitis B virus (HBV) (e.g., entecavir), and HCV (e.g., sofosbuvir). Drugs that inhibit viral proteases are used to treat HCV[149] and HIV[150] infection. These drugs block the proteolytic processing of viral precursor polyproteins and serve as potent inhibitors of replication. Other viral enzymes also serve as targets for antiviral therapy. The influenza virus neuraminidase is required for the release of progeny influenza virus particles from infected cells. Oseltamivir, peramivir, and zanamivir bind the neuraminidase catalytic site and efficiently inhibit the enzyme.[151] These drugs have been used in the prophylaxis and treatment of influenza virus infection.[152]

Better understanding of viral replication strategies and mechanisms of virus-induced cell killing is paving the way for the rational design of new antiviral therapeutics. One of the most exciting approaches to the development of antiviral agents is the use of high-resolution x-ray crystallography and molecular modeling to optimize interactions between these inhibitory molecules and their target viral proteins. Such structure-based drug design has led to the development of synthetic peptides (e.g., enfuvirtide) that inhibit HIV entry by blocking gp41-mediated membrane fusion.[153] Other vulnerable steps in HIV replication are targets of drugs approved for patient treatment, including entry inhibitors that interfere with gp120 binding to CCR5[154] and agents that prevent proviral integration into cellular DNA through inhibition of viral integrase activity[155] (see Chapter 128). Viral replication complexes have been successfully targeted in the development of highly effective regimens for treatment of HCV infection. Inhibitors of the HCV NS5A protein disrupt its essential function in the formation of membranous structures that scaffold viral RNA replication and virion assembly. In a combination antiviral approach analogous to the treatment of HIV infection, NS5A inhibitors are coadministered with drugs that specifically target the HCV RdRp and NS3-4A protease, which creates a high genetic barrier to resistance through interference with multiple steps in the viral replication program. Notably, combination therapy is rapidly becoming a matter of first principles in the research and development of new antiviral drugs and durable therapeutic strategies (see Chapter 47).

Despite promising advances in rational antiviral drug design, current therapeutic approaches to some viral infections rely heavily on compounds with less specific mechanisms of action. One such agent, interferon-α (IFN-α), efficiently inhibits a broad spectrum of viruses and is secreted by diverse cell types as part of the host innate immune response. Recombinant IFN-α is used to treat certain patients with HBV infections (see Chapter 145). Ribavirin, a synthetic guanosine analogue, inhibits the replication of many RNA- and DNA-containing viruses through complex mechanisms involving inhibition of viral RNA synthesis and disturbances in intracellular pools of guanosine triphosphate.[156,157] This drug is used to treat some HCV infections and is sometimes administered in aerosolized form to treat respiratory syncytial virus (RSV) lower respiratory tract infection in hospitalized children and in severely ill and immunocompromised persons. Ribavirin therapy reduces the mortality associated with certain viral hemorrhagic fevers, such as that caused by Lassa virus.[158] Broader-spectrum therapies exemplified by IFN-α and ribavirin are considered for use against emerging pathogens and other susceptible viruses for which biochemical and structural information is insufficient to design high-potency agent-specific drugs.

VIRUS-HOST INTERACTION

One of the most formidable challenges in virology is to apply knowledge gained from studies of virus-cell interactions in tissue culture systems to an understanding of how viruses interact with host organisms to cause disease. Virus-host interactions are often described in terms of pathogenesis and virulence. *Pathogenesis* is the process whereby a virus

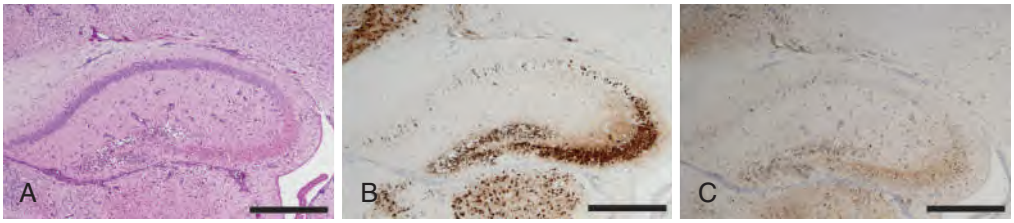

FIG. 131.5 Reovirus-induced apoptosis in the murine central nervous system. Consecutive sections of the hippocampus prepared from a newborn mouse 10 days after intracranial inoculation with reovirus. Cells were stained with (A) hematoxylin and eosin, (B) reovirus antiserum to detect viral antigen, and (C) antiserum to detect the activated form of apoptosis protease caspase-3. Cells that stain positive for reovirus antigen or activated caspase-3 contain a dark precipitate in the cytoplasm, including neuronal processes. Scale bars, 100 μm. *(Modified from Danthi P, Coffey CM, Parker JS, et al. Independent regulation of reovirus membrane penetration and apoptosis by the μ1 Φ domain.* PLoS Pathog. *2008;4:e1000248.)*

interacts with its host in a discrete series of stages to produce disease. *Virulence* is the capacity of a virus to produce disease in a susceptible host. Virulence is often measured in terms of the quantity of virus required to cause illness or death in a predefined fraction of hosts infected with the virus. Virulence is dependent on viral and host factors and must be measured using carefully defined conditions (e.g., virus strain, dose, and route of inoculation; host species, age, and immune status). In many cases, it has been possible to identify roles played by individual viral and host proteins at specific stages in viral pathogenesis and to define the importance of these proteins in viral virulence. Recently, the concept of microbial "pathogenic potential" has been introduced as a metric of its capacity to cause disease.[159] Pathogenic potential attempts to relate disease expression in the form of symptoms or death to organism dose and communicability.

Entry

The first step in the process of virus-host interaction is the exposure of a susceptible host to viable virus under conditions that promote infection (Fig. 131.6). Infectious virus may be present in respiratory droplets or aerosols, in contaminated food or water, or in a body fluid or tissue (e.g., blood, saliva, urine, semen, or a transplanted organ) to which the susceptible host is exposed. In some cases, the virus is inoculated directly into the host through the bite of an animal vector or through the use of a contaminated needle. Infection also can be transmitted from mother to infant through virus that has infected the placenta or birth canal or by virus in breast milk. In some cases, acute viral infections result from the reactivation of endogenous latent virus (e.g., reactivation of HSV giving rise to herpes labialis) rather than de novo exposure to exogenous virus.

Exposure of respiratory mucosa to virus by direct inoculation or inhalation is an important route of viral entry into the host. A simple cough can generate up to 10,000 small, potentially infectious aerosol particles, and a sneeze can produce nearly 2 million. The distribution of these particles depends on a variety of environmental factors, the most important of which are temperature, humidity, and air currents. In addition to these factors, particle size is an important determinant of particle distribution. In general, smaller particles remain airborne longer than larger ones. Particle size also contributes to particle fate after inhalation. Larger particles (>6 μm) are generally trapped in the nasal turbinates, whereas smaller particles may ultimately travel to the alveolar spaces of the lower respiratory tract.

Fecal-oral transmission represents an additional important route of viral entry into the host. Food, water, or hands contaminated by infected fecal material can facilitate the entry of a virus via the mouth into the gastrointestinal tract, the environment of which requires viruses that infect by this route to have certain physical properties. Viruses capable of enteric transmission must be stable to gastric acidity and resistant to bile salts. Because conditions in the stomach and intestine are destructive to the lipids contained in viral envelopes, most viruses that spread by the fecal-oral route are nonenveloped. Interesting to note, many viruses that enter the host via the gastrointestinal tract require proteolysis of certain capsid components to infect intestinal cells productively. Treatment of mice with inhibitors of intestinal proteases blocks infection by reovirus[160] and rotavirus,[161] which demonstrates the critical importance of proteolysis in the initiation of enteric infection by these viruses. The host microbiota is essential for infection by some viruses.[162–164]

To produce systemic disease, a virus must cross the mucosal barrier that separates the luminal compartments of the respiratory, gastrointestinal, and genitourinary tracts from the host's parenchymal tissues. Studies with reovirus illustrate one strategy used by viruses to cross mucosal surfaces to invade the host after entry into the gastrointestinal tract.[165,166] After oral inoculation of mice, reovirus adheres to the surface of intestinal microfold cells (M cells) that overlie collections of intestinal lymphoid tissue (Peyer's patches). In electron micrographs, reovirus virions can be followed sequentially as they are transported within vesicles from the luminal to the subluminal surface of M cells. Virions subsequently appear within Peyer's patches and then spread to regional lymph nodes and extraintestinal lymphoid organs such as the spleen. A similar pathway of spread is used by poliovirus[167] and HIV,[168] suggesting that M cells represent an important portal for viral invasion of the host after entry into the gastrointestinal tract.

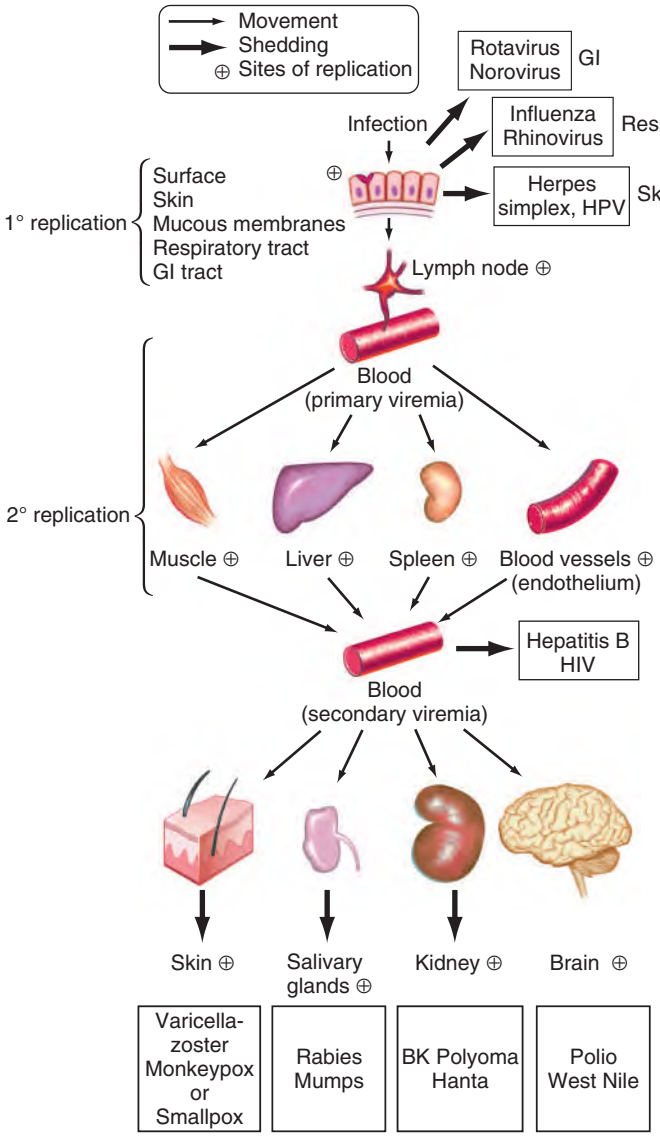

FIG. 131.6 Entry and spread of viruses in human hosts. Some major steps in viral spread and invasion of target organs are shown, represented by example viruses. Neural spread is not illustrated. *GI,* Gastrointestinal; *Resp.,* respiratory; *HIV,* human immunodeficiency virus; *HPV,* human papillomavirus. *(Modified from Nathanson N, Tyler KL. Entry, dissemination, shedding, and transmission of viruses. In: Nathanson N, ed.* Viral Pathogenesis. *Philadelphia: Lippincott-Raven; 1997:13–33.)*

Spread

Once a virus has entered the host, it can replicate locally or spread from the site of entry to distant organs to produce systemic disease (see Fig. 131.6). Classic examples of localized infections in which viral entry and replication occur at the same anatomic site include respiratory infections caused by influenza virus, rhinovirus, and RSV; enteric infections produced by norovirus and rotavirus; and dermatologic infections caused by HSV (cold sores and genital ulcers) and HPV (warts). Other viruses spread to distant sites in the host after primary replication at sites of entry. For example, poliovirus spreads from the gastrointestinal tract to the central nervous system (CNS) to produce meningitis, encephalitis, or poliomyelitis. Measles virus and varicella-zoster virus (VZV) enter the host through the respiratory tract and then spread to lymph nodes, skin, and viscera. Pathobiologic definitions of viruses based on spread potential have begun to blur amid accumulating evidence that model agents of localized infection may disseminate to distant sites. For example,

rotavirus, an important cause of acute gastroenteritis in children, replicates vigorously in villous tip epithelial cells of the small intestine but also frequently invades the bloodstream, the clinical significance of which is unclear.[169] Influenza virus is another case in point; viral RNA in blood is detected at a substantial frequency in hematopoietic cell transplant recipients and correlates with more severe disease and increased mortality.[170] Reports of rhinovirus viremia have begun to reshape thinking about the pathogenesis and disease spectrum of rhinovirus infections, which probably account for more episodes of serious respiratory illness than traditionally appreciated.[171,172]

Release of some viruses occurs preferentially from the apical or basolateral surface of polarized cells, such as epithelial cells. In the case of enveloped viruses, polarized release is frequently determined by preferential sorting of envelope glycoproteins to sites of viral budding. Specific amino-acid sequences in these viral proteins direct their transport to a particular aspect of the cell surface.[173,174] Polarized release of virus at apical surfaces may facilitate local spread of infection, whereas release at basolateral surfaces may facilitate systemic invasion by providing virus access to subepithelial lymphoid, neural, or vascular tissues.

Many viruses use the bloodstream to spread in the host from sites of primary replication to distant target tissues (see Fig. 131.6). In some cases, viruses may enter the bloodstream directly, such as during a blood transfusion or via an arthropod bite. More commonly, viruses enter the bloodstream after replication at some primary site. Important sites of primary replication preceding hematogenous spread of viruses include Peyer's patches and mesenteric lymph nodes for enteric viruses, bronchoalveolar cells for respiratory viruses, and subcutaneous tissue and skeletal muscle for alphaviruses and flaviviruses. In the case of reovirus, infection of endothelial cells leads to hematogenous dissemination in the host.[175,176]

Pioneering studies by Fenner with mousepox (ectromelia) virus suggested that an initial low-titer viremia (primary viremia) serves to seed virus to a variety of intermediate organs, where a period of further replication leads to a high-titer viremia (secondary viremia) that disseminates virus to the ultimate target organs (Fig. 131.7).[177] It is often difficult to discriminate primary and secondary viremias in naturally occurring viral infections. However, replication of many viruses in reticuloendothelial organs (e.g., liver, spleen, lymph nodes, and bone marrow), muscle, fat, and even vascular endothelial cells can play an important role in maintaining viremia.[176]

Viruses that reach the bloodstream may travel free in plasma (e.g., enteroviruses and togaviruses) or in association with specific blood cells.[178] A number of viruses are spread hematogenously by macrophages (e.g., CMV, HIV, and measles virus) or lymphocytes (e.g., CMV, EBV, HIV, HTLV, and measles virus). Although many viruses have the capacity to agglutinate erythrocytes in vitro (a process called hemagglutination), only in exceptional cases (e.g., Colorado tick fever virus) are erythrocytes used to transport virus in the bloodstream.

The maintenance of viremia depends on an interplay among factors that promote virus production and those that favor viral clearance. A number of variables that affect the efficiency of virus removal from plasma have been identified. In general, the larger the viral particle, the more efficiently it is cleared. Viruses that induce high titers of neutralizing antibodies are more efficiently cleared than those that do not induce humoral immune responses. Finally, phagocytosis of virus by cells in the host reticuloendothelial system can contribute to viral clearance.

A major pathway used by viruses to spread from sites of primary replication to the nervous system is through nerves. Numerous diverse viruses, including Borna disease virus, HSV, poliovirus, rabies virus, reovirus, and Venezuelan equine encephalitis (VEE) virus, are capable of neural spread. Several of these viruses accumulate at the neuromuscular junction after primary replication in skeletal muscle.[179,180] HSV appears to enter nerve cells via receptors that are located primarily at synaptic endings rather than on the nerve cell body.[181] Spread to the CNS by HSV,[182] rabies virus,[179,180] and reovirus[183,184] can be interrupted by scission of the appropriate nerves or by chemical agents that inhibit axonal transport. Neural spread of some of these viruses occurs through the microtubule-based system of fast axonal transport.[185]

Viruses are not limited to a single route of spread. VZV, for example, enters the host by the respiratory route and then spreads from respiratory epithelium to the reticuloendothelial system and skin via the bloodstream. Infection of the skin produces the characteristic exanthem of chickenpox. The virus subsequently enters distal termini of sensory neurons and travels to dorsal root ganglia, where it establishes latent infection. Reactivation of VZV from latency results in transport of the virus in sensory nerves to skin, where it gives rise to vesicular lesions in a dermatomal distribution characteristic of zoster or *shingles*.

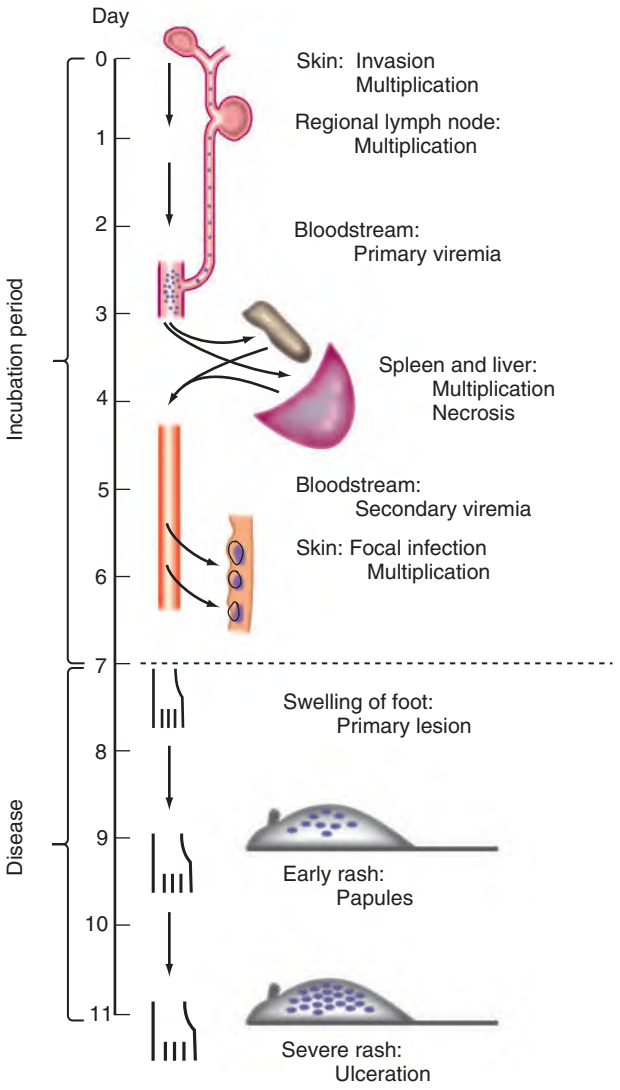

FIG. 131.7 Pathogenesis of mousepox virus infection. Successive waves of viremia are shown to seed the spleen and liver and then the skin. *(From Fenner F. Mousepox [infectious ectromelia of mice]: a review.* J Immunol. *1949;63:341–373.)*

Poliovirus also is capable of spreading by hematogenous and neural routes. Poliovirus is generally thought to spread from the gastrointestinal tract to the CNS via the bloodstream, although it has been suggested that the virus may spread via autonomic nerves in the intestine to the brainstem and spinal cord.[186,187] This hypothesis has been supported by experiments using transgenic mice expressing the human poliovirus receptor.[188] When these mice are inoculated with poliovirus intramuscularly in the hind limb, virus does not reach the CNS if the sciatic nerve ipsilateral to the site of inoculation is transected.[189] Once poliovirus reaches the CNS, axonal transport is the major route of viral dissemination. Similar mechanisms of spread may be used by other enteroviruses.

Tropism

The capability of a virus to infect a distinct group of cells in the host is referred to as *tropism*. For many viruses, tropism is determined by

the availability of virus receptors on the surface of a host cell. This concept was first appreciated in studies of poliovirus when it was recognized that the capacity of the virus to infect specific tissues paralleled its capacity to bind homogenates of the susceptible tissues in vitro.[190] The importance of receptor expression as a determinant of poliovirus tropism was conclusively demonstrated by showing that cells not susceptible for poliovirus replication could be made susceptible by recombinant expression of the poliovirus receptor.[191] In addition to the availability of virus receptors, tropism also can be determined by postattachment steps in viral replication, such as the regulation of viral gene expression. For example, some viruses contain genetic elements, termed *enhancers,* which act to stimulate transcription of viral genes.[192,193] Some enhancers are active in virtually all types of cells, whereas others show exquisite tissue specificity. The promoter-enhancer region of John Cunningham (JC) polyomavirus is active in cultured human glial cells but not in HeLa cervical epithelial cells.[194] Cell-specific expression of the JC virus genome correlates well with the capacity of this virus in immunocompromised persons to produce progressive multifocal leukoencephalopathy, a disease in which JC virus infection is limited to oligodendroglia in the CNS.

Specific steps in virus-host interaction, such as the route of entry and pathway of spread, also can strongly influence viral tropism. For example, encephalitis viruses such as VEE virus are transmitted to humans by insect bites. These viruses undergo local primary replication and then spread to the CNS by hematogenous and neural routes.[195] After oral inoculation, VEE virus is incapable of primary replication and spread to the CNS, illustrating that tropism can be influenced by the site of entry into the host. Influenza virus buds exclusively from the apical surface of respiratory epithelial cells,[196] which may limit its capacity to spread within the host and infect cells at distant sites.

A wide variety of host factors influence viral tropism. These include age, nutritional status, immune responsiveness, and certain genetic polymorphisms that affect susceptibility to viral infection. Age-related susceptibility to infection is observed for many viruses, including reovirus,[197,198] rotavirus,[199,200] and RSV.[201–203] The increased susceptibility in young children to these viruses may in part be due to immaturity of the immune response but also may be related to intrinsic age-specific factors that enhance host susceptibility to infection. Nutritional status is a critical determinant of the tropism and virulence of many viruses. For example, persons with vitamin A deficiency have enhanced susceptibility to measles virus infection.[204,205] Similarly, the outcome of most viral infections is strongly linked to the immune competence of the host.

The genetic basis of host susceptibility to viral infections is complex. Studies with inbred strains of mice indicate that genetic variation can alter susceptibility to viral disease through a variety of mechanisms.[206] These can involve differences in immune responses, variability in the ability to produce antiviral mediators such as IFN, and differential expression of functional virus receptors. Polymorphisms in the expression of chemokine receptor CCR5, which serves as a coreceptor for HIV,[52–54] are associated with alterations in susceptibility to HIV infection.[207,208] Furthermore, a single amino-acid polymorphism in the human rhinovirus C receptor, CDHR3, influences its abundance at the cell surface and the subsequent severity of viral infection.[209]

Added to effects of host genetic diversity, the intrinsic nonviral (i.e., principally bacterial) microbiota has come to attention as an important variable in host susceptibility to viral infection. Local microbial flora may exert a positive or negative influence on viral infectivity through mechanisms that may involve direct interactions between viruses and bacterial products or intermediary functions of the host immune system.[210] Notable examples of viral infections modulated by commensal microbes include mouse mammary tumor virus,[163] norovirus,[164] poliovirus,[162,214] and reovirus.[162]

Persistent Infections

Many viruses are capable of establishing persistent infections, of which two types are recognized: chronic and latent. *Chronic viral infections* are characterized by continuous production of virus for prolonged periods of time. Congenital infections with rubella virus and CMV and persistent infections with HBV and HCV are examples of chronic viral infections. *Latent viral infections* are characterized by maintenance of the viral genome in host cells in the absence of viral replication. Herpesviruses and retroviruses can establish latent infections. The distinction between chronic and latent infections is not readily apparent for some viruses, such as HIV, which can establish both chronic and latent infections in the host.[216–218] Viruses capable of establishing persistent infections must have a means of evading the host immune response and a mechanism of attenuating their virulence. For example, HCV[219] and HIV[220–222] are capable of extensive antigenic variation resulting in escape from neutralizing antibody responses by the host.

Several viruses encode proteins that directly attenuate the host immune response; for example, the adenovirus E3/19K protein[223] and CMV US11 gene product[224] block cell surface expression of MHC class I proteins, resulting in diminished presentation of viral antigens to cytotoxic T lymphocytes (CTLs). The poxviruses encode a variety of immunomodulatory molecules including CrmA, which blocks T-cell–mediated apoptosis of virus-infected cells.[225] In some cases (e.g., the CNS), preferential sites for persistent viral infections are not readily accessible by the immune system,[226] which may favor establishment of persistence. The central position and potent effects of innate immune responses in the control of viral infections, mediated primarily by the type 1 IFN system, has driven myriad survival adaptations and countermeasures among virtually all known human viruses. Specialized proteins or nucleic acids may function in viral antagonism of innate immunity, or viruses may adopt a passive strategy of sequestering provocative signals from cellular sensors of microbial invasion.[227,228]

Viruses and Cancer

Several viruses produce disease by promoting malignant transformation of host cells. Work by Peyton Rous with an avian retrovirus was the first to demonstrate that viral infections can cause cancer.[229] Rous sarcoma virus encodes an oncogene, v-*src,* which is a homologue of a cellular proto-oncogene, c-*src.*[230,231] Cells infected with Rous sarcoma virus become transformed.[232–236] Several viruses are associated with malignancies in humans. EBV is associated with many neoplasms, including Burkitt lymphoma, Hodgkin disease, large B-cell lymphoma, leiomyosarcoma, nasopharyngeal carcinoma, and multicentric Castleman disease. HBV and HCV are associated with hepatocellular carcinoma. HPV is associated with cervical cancer and a variety of anogenital and esophageal neoplasms. Kaposi sarcoma–associated herpesvirus is associated with Kaposi sarcoma and primary effusion lymphoma in persons with HIV infection.

Often, linkage of a virus to a particular neoplasm can be attributed to transforming properties of the virus itself. For example, EBV encodes several latency-associated proteins that are responsible for immortalization of B cells; these proteins likely play crucial roles in the pathogenesis of EBV-associated malignancies.[237] Similarly, HPV encodes the E6 and E7 proteins that block apoptosis[111–113] and induce cell cycle progression,[109,110] respectively. It is hypothesized that unregulated expression of these proteins induced by the aberrant integration of the HPV genome into host DNA is responsible for malignant transformation.[238] The tumorigenicity of polyomaviruses is mediated by a family of viral proteins known as tumor (T) antigens. Reminiscent of the HPV E6 and E7 proteins, T antigens induce cell cycling and block the ensuing cellular apoptotic response to unscheduled cell division.[239] The normally episomal polyomavirus genome becomes integrated into cellular DNA during neoplastic transformation of nonpermissive cells unable to support the entire viral replication program, which would otherwise culminate in cell death. Discovery of a human polyomavirus clonally integrated into cells of an aggressive form of skin cancer, Merkel cell carcinoma,[240] substantiates the long-standing suspicion that polyomaviruses can promote neoplasia in humans.

In other cases, mechanisms of malignancy triggered by viral infection are less clear. HCV is an RNA-containing virus that lacks reverse transcriptase and a means of viral genome integration. However, chronic infection with HCV is strongly associated with hepatocellular cancer.[241] It is possible that increased cell turnover and inflammatory mediators elicited by chronic HCV infection increase the risk of genetic damage,

which results in malignant transformation. Some HCV proteins also may contribute to neoplasia. For example, the HCV core protein can protect cells against apoptosis induced by a variety of stimuli, including tumor necrosis factor-α (TNF-α).[242]

VIRAL VIRULENCE DETERMINANTS

Viral surface proteins involved in attachment and entry influence the virulence of diverse groups of viruses. For example, polymorphisms in the attachment proteins of influenza virus,[243,244] polyomavirus,[245] reovirus,[246] rotavirus,[247] and VEE virus[248] are strongly linked to virulence and can be accurately termed *virulence determinants.* Viral attachment proteins can serve this function by altering the affinity of virus-receptor interactions or modulating the kinetics of viral disassembly. Important to note, sequences in viral genomes that do not encode protein also can influence viral virulence. Mutations that contribute to the attenuated virulence of the Sabin strains of poliovirus are located in the 5′ nontranslated region of the viral genome.[249] These mutations attenuate poliovirus virulence by altering the efficiency of viral protein synthesis. In another variation on the theme of virulence regulation by nontranslated RNA, a long noncoding RNA, known as polyadenylated nuclear (PAN) RNA, produced by Kaposi sarcoma–associated herpesvirus downregulates the host antiviral response and is required for induction of lytic replication from latency.[250]

A number of viruses encode proteins that enhance virulence by modulation of host immune responses. Illustrative examples include the influenza A NS1 protein, which interferes with activation of cellular innate immune responses to viral infection,[251] and translation products of the adenovirus E3 transcriptional unit, which serve to prevent cytotoxic T-cell recognition of virus-infected cells and block immunologically activated signaling pathways that lead to death of infected cells.[223,252] In many cases, these proteins are dispensable for viral replication in cultured cells. In this way, immunomodulatory viral virulence determinants are analogous to classical bacterial virulence factors such as various types of secreted toxins.

HOST RESPONSES TO INFECTION

The immune response to viral infection involves complex interactions among leukocytes, nonhematopoietic cells, signaling proteins, soluble proinflammatory mediators, antigen-presenting molecules, and antibodies. These cells and molecules collaborate in a highly regulated fashion to limit viral replication and dissemination through recognition of broadly conserved molecular signatures, followed by virus-specific adaptive responses that further control infection and establish antigen-selective immunologic memory. The innate antiviral response is a local, transient, antigen-independent, perimeter defense strategically focused at the site of virus incursion into an organ or tissue. Mediated by ancient families of membrane-associated and cytosolic molecules known as *pattern recognition receptors* (PRRs), the innate immune system detects pathogen-associated molecular patterns (PAMPs), which are fundamental structural components of microbial products including nucleic acids, carbohydrates, and lipids.[253] Viral PAMPs in the form of single-stranded (ss)RNA, dsRNA, RNA 5′ triphosphate, and DNA evoke the innate immune response through two groups of PRRs: the transmembrane Toll-like receptors (TLRs) and the cytosolic and nuclear nucleic acid sensors. The latter include retinoic acid inducible gene-I (RIG-I)-like receptors and DNA sensors such as cyclic GMP-AMP synthase.[254] Nucleic acid binding by PRRs activates signaling pathways leading to the production and extracellular release of IFN-α, IFN-β, and proinflammatory cytokines such as IL-1β and IL-18. IFN-α and IFN-β engage the cell surface IFN-α/β receptor and thereby mediate expression of hundreds of gene products that corporately suppress viral replication and establish an intracellular antiviral state in neighboring uninfected cells. Well-described IFN-inducible gene products include the latent enzymes dsRNA-dependent protein kinase (PKR) and 2′,5′-oligoadenylate synthetase (OAS), both of which are activated by dsRNA.[255] PKR inhibits the initiation of protein synthesis through phosphorylation of translation initiation factor eIF2α. The 2′,5′-oligoandenylates generated by OAS bind and activate endoribonuclease RNase L, which degrades viral mRNA. In addition to mediating an intracellular antiviral state, IFN-α/β also stimulates the antigen-independent destruction of virus-infected cells by a specialized population of lymphocytes known as natural killer (NK) cells.[256] Important to note, IFNs bridge innate and adaptive antiviral immune responses through multiple modes of action, which include enhancing viral antigen presentation by class I MHC proteins,[257] promoting the proliferation of MHC class I–restricted $CD8^+$ CTLs,[258] and facilitating the functional maturation of dendritic cells.[259] Proinflammatory mediators IL-1β and IL-18 pleiotropically stimulate and amplify the innate immune response through induction of other inflammatory mediators, immune cell activation, and migration of inflammatory cells into sites of infection.[260] These molecules perform essential functions in host antiviral defense.[261]

The adaptive immune response confers systemic and enduring pathogen-selective immunity through expansion and functional differentiation of viral antigen–specific T and B lymphocytes. Having both regulatory and effector roles, T lymphocytes are centrally positioned in the scheme of adaptive immunity. The primary cell type involved in the resolution of acute viral infection is the $CD8^+$ CTL, which induces lethal proapoptotic signaling in virus-infected cells on recognition of endogenously produced viral protein fragments presented by cell surface MHC class I molecules. Less frequently, $CD4^+$ T cells, which recognize MHC class II–associated viral oligopeptides processed from exogenously acquired proteins, also demonstrate cytotoxicity against viral antigen-presenting cells.[262] The usual function of $CD4^+$ T lymphocytes is to orchestrate and balance cell-mediated (CTL) and humoral (B lymphocyte) responses to infection. Several classes of helper T (Th) $CD4^+$ cell subsets have been defined based on characteristic patterns of cytokine secretion, gene-expression profiles, and effector activities.[263,264,265,266,267] Th1, Th2, Th9, Th17, Th22, T-regulatory (Treg), and T-follicular helper (Tfh) cells comprise distinguishable mature populations derived from undifferentiated $CD4^+$ precursor lymphocytes. Th1 and Th2 lymphocytes are usually associated with the development of cell-mediated and humoral responses, respectively, to viral infection. Understanding continues to evolve about the exact roles of other Th subsets in viral disease and antiviral immunity. For certain persistent viral infections, such as those caused by HIV and HSV, Treg cells might exacerbate disease through suppression of CTLs or, paradoxically, might ameliorate illness by attenuating immune-mediated cell and tissue injury.[265] Tfh cells promote differentiation of antigen-specific memory B lymphocytes and plasma cells within germinal centers.[266] Therefore Tfh cells likely occupy a central place in the humoral response to viral infection and vaccination. Although Tfh cell functions are not unique to antiviral responses, chronic viral infections including HBV and HIV appear to stimulate proliferation of these cells.[268,269]

The primacy of cell-mediated immune responses in combating viral infections is revealed by the extreme vulnerability of individuals to chronic and life-threatening viral diseases when cellular immunity is dysfunctional. Those with acquired immunodeficiency syndrome (AIDS) exemplify the catastrophic consequences of collapsing cell-mediated immunity; progressive multifocal leukoencephalopathy caused by JC polyomavirus, along with severe mucocutaneous and disseminated CMV, HSV, and VZV infections, are frequent complications of diminishing numbers of $CD4^+$ T cells. Similarly, iatrogenic cellular immunodeficiency associated with hematopoietic stem cell and solid-organ transplantation or antineoplastic treatment regimens predisposes to severe, potentially fatal infections with herpesviruses and respiratory viral pathogens such as adenovirus, coronavirus, influenza virus, PIV, and RSV,[270,271] all of which normally produce self-limited illness in immunocompetent hosts. Prevention and management of serious viral respiratory infections are significant challenges in myelosuppression units because of the communicability of respiratory viruses and paucity of effective drugs to combat these ubiquitous agents. Individuals with significantly impaired cell-mediated immunity also are at increased risk for enhanced viral replication and systemic disease after immunization with live-attenuated viral vaccines (e.g., measles, mumps, and rubella [MMR] and VZV vaccines). Hence, live viral vaccines are generally contraindicated for immunocompromised persons (see Chapter 316). TNF-α inhibitor therapy, increasingly used to manage a variety of inflammatory and rheumatologic diseases, enhances the risk of HBV reactivation, with potentially life-threatening consequences.[272]

Preventive and interventional HBV treatment strategies are necessary to circumvent complications of uncontrolled viral replication in these patients.

In contrast to cell-mediated immune mechanisms, humoral responses are usually not a determinative factor in the resolution of primary viral infections. One notable exception is a syndrome of chronic enteroviral infections in the setting of agammaglobulinemia.[273] However, for most human viral pathogens, the presence of antibody is associated with protection against initial infection in vaccinees or reinfection in hosts with a history of natural infection.[274] Longitudinal studies indicate that levels of protective serum antibodies (induced by natural infection or immunization) to common viruses, including EBV and measles, mumps, and rubella viruses, are remarkably stable, with calculated antibody half-lives ranging from several decades to thousands of years.[275] The protective role of antibodies on secondary exposure is frequently explained as interruption of viremic spread when a hematogenous phase is involved, such as occurs with measles, mumps, and rubella viruses, poliovirus, VZV, and most arboviruses. Nevertheless, most human viruses, excluding arthropod-transmitted agents, enter their hosts by penetration of a mucosal barrier, frequently undergoing primary replication in mucosal epithelium or adjacent lymphoid tissues. Neutralizing immunoglobulin A (IgA) exuded onto mucosal epithelial surfaces may protect against primary infection at this portal of viral entry. A classic example is gut mucosal immunity induced by orally administered Sabin poliovirus vaccine containing live-attenuated virus. Secretory IgA against poliovirus blocks infection at the site of primary replication and consequently interrupts the chain of viral transmission, although fully virulent revertant viruses arise at regular frequency in vaccine recipients, who may develop disease and also transmit revertant strains to nonimmune individuals.[276] Clinical and experimental studies of immunity to HIV have led to the recognition that resident immune responses at exposed mucosal surfaces are likely critical components of host resistance to primary HIV infection, and achievement of potent mucosal immunity has emerged as an important consideration for the design of candidate HIV vaccines.[277] Despite the appearance of serum neutralizing antibodies to HIV several weeks after infection, viral eradication is thwarted by selection of neutralization-resistant variant strains from a mutant pool, which is perpetually replenished because of extreme plasticity within neutralization determinants on the viral envelope glycoproteins.[278] Identification of epitopes bound by broadly neutralizing antiviral antibodies has provided potential new targets for structure-based vaccine design[279]; such a strategy has yielded a promising RSV vaccine candidate.[280]

Protection against viral infection by serum immunoglobulins is often correlated with antibody-mediated neutralization of viral infectivity in cultured cells. Antibodies interrupt the viral life cycle at early steps, which may include cross-linking virion particles into noninfectious aggregates, steric hindrance of receptor engagement, and interference with viral disassembly.[281] It is presumed that virus neutralization in cell culture by human serum is reflective of antibody activity in the intact host, but the mechanistic basis of infection blockade and disease prevention by antibodies in vivo is difficult to define precisely. For example, exclusively in vivo functions of the humoral antiviral response include Fc-mediated virion phagocytosis[282,283] and antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC responses require effectors from both the innate and adaptive systems, NK cells and antibodies, respectively.[284] The basis of ADCC is FcγRIIIA (CD16) receptor-dependent recognition by NK cells of virus-specific IgG bound to antigens expressed on the surface of infected cells, leading to release of perforin and granzymes from NK cells that eventuate in target cell apoptosis. Neutrophils, lymphocytes, and macrophages also possess Fc receptors and may participate in ADCC. New vaccine strategies are being developed to elicit these responses.

Although exuberant innate and adaptive antiviral immune responses are essential to restoration and preservation of normal host physiology in the face of viral infection, viruses paradoxically can undermine delicate systems of immune homeostasis. One such example is celiac disease, a complex T-cell-mediated intestinal disorder with an autoimmune component characterized by an inflammatory immune response to dietary gluten that occurs exclusively in individuals expressing human leukocyte antigen (HLA) DQ2 or DQ8. Approximately 30% to 45% of the US population carries a DQ2 or DQ8 allele, yet <1% of the population develops the disease. This observation suggests that additional environmental factors, such as viral infections, contribute to disease induction in genetically susceptible individuals. Infection of mice with some (but not all) strains of reovirus abrogates tolerance to fed antigen. Interferon regulatory factor 1, a transcription factor important in the antiviral immune response, is a key mediator of virus-induced loss of tolerance to fed antigen and celiac disease development. Moreover, humans with celiac disease have higher titers of antireovirus antibodies relative to unaffected controls, suggesting that reovirus may be an environmental trigger for the disease.[285]

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The complete reference list is available online at Expert Consult.

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132 Orthopoxviruses Vaccinia (Smallpox Vaccine), Variola (Smallpox), Monkeypox, and Cowpox

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SHORT VIEW SUMMARY

Definition
- Orthopoxvirus infections can cause a spectrum of febrile rash illnesses in humans, ranging from fairly benign, localized skin infections to severe systemic infections.
- Vaccinia (smallpox vaccine), variola (smallpox), monkeypox, cowpox, and several newly discovered orthopoxvirus species are known to cause human disease.

Epidemiology
- Most orthopoxvirus infections are zoonotic, with humans serving as accidental hosts.
- Vaccinia virus continues to be used as a vaccine as well as a subject and tool for biomedical research, and it causes sporadic disease in vaccinees, contacts of vaccinees, laboratory workers, and farmworkers with occupational exposure to cattle.
- Variola, the causative agent of smallpox, caused significant morbidity and mortality worldwide before its eradication in 1980.
- Monkeypox virus causes intermittent human infections, primarily in Central Africa and West Africa, although isolated outbreaks have been identified in the United States and Sudan.
- Human cowpox virus infection was historically associated with occupational exposure to cattle; however, more recently, pet rats, pet cats, and zoo and circus elephants have been implicated.

Microbiology
- Orthopoxviruses are a group of large, complex, double-stranded DNA viruses that replicate in the cytoplasm of the host cell.

Diagnosis
- Diagnostic laboratory testing for orthopoxvirus infections can include polymerase chain reaction, viral culture, and electron microscopy of rash lesion material as well as serologic testing of serum.

Therapy
- Vaccinia immune globulin intravenous is licensed for treatment of certain complications of vaccinia vaccine administration and is available through the US Centers for Disease Control and Prevention.
- Tecovirimat is an antiviral compound licensed for treatment of human smallpox disease and is available through the Centers for Disease Control and Prevention.
- The development and evaluation of vaccines and therapeutics for orthopoxvirus infections is an active area of research.

Prevention
- Orthopoxviruses induce cross-reactive antibodies that protect against infection from other orthopoxvirus species.
- Vaccination with smallpox vaccine (i.e., vaccinia virus) can be used to protect individuals at high risk of orthopoxvirus disease.
- Other control measures focus on educational outreach to decrease the risk of exposure to likely zoonotic vectors.

BACKGROUND

The genus *Orthopoxvirus* belongs to the family of Poxviridae, a group of large, complex, double-stranded DNA viruses that replicate in the cytoplasm of the host cell and are defined by their genomic, structural, and antigenic similarities.[1,2] Humans can be infected by members of multiple poxvirus genera, but they are usually accidental hosts. Most human infections are zoonotic, and animal exposure and geographic location give clues to the etiologic agent. The orthopoxvirus variola, however, is a selective human pathogen. Variola is the causative agent of smallpox, which in 1980 was declared by the World Health Organization (WHO) to be eradicated worldwide. Other orthopoxviruses known to infect humans are cowpox, vaccinia, and monkeypox. New orthopoxviruses that cause localized human disease have been identified in the past 5 years (AK2015, NY_v014; discussed later). Species of poxvirus genera that infect humans other than within the *Orthopoxvirus* genus are discussed in Chapter 133. Comprehensive book chapter reviews and monographs on poxvirus virology are available.[3–5]

The ability of sera raised against one orthopoxvirus species to cross-neutralize another species is one of the fundamental reasons for cross-protection provided with vaccination. Vaccinia virus is the orthopoxvirus species now characterized as the constituent of smallpox vaccine. Other orthopoxviruses used as smallpox vaccines include horsepox virus and cowpox virus.[6] Because of the continued characterization of orthopoxviruses as causes of human illness and in part because of the concern that variola could be used as an agent of bioterrorism or as a biological weapon, there is continued interest and research on orthopoxviruses and poxviruses. The demonstration that orthopoxviruses can be created *de novo* using synthetic biology techniques has added a new dimension to these concerns.[7] Research on vaccinia is targeting fundamental viral properties and use of the virus as a (vaccine) vector as well as potential use as an oncolytic virus.[8,9] The emergence in 2003 of human clinical illness caused by monkeypox in the United States and ongoing disease in Central Africa, renewed reports of human monkeypox in West Africa in 2017, continued recurrence of human vaccinia infections in Latin American countries, and cowpox infections in Eurasia have also increased the need for knowledge of, and treatments for, these viral pathogens. Variola virus and the Congo Basin clade of monkeypox virus are considered select agents by the US Department of Health and Human Services and the US Department of Agriculture; these considerations impose additional restrictions on use in research laboratories and on reporting on discovery of these agents in clinical specimens.[10]

MORPHOLOGY AND CHEMICAL STRUCTURE

The poxviruses described in this chapter and Chapter 133 belong to the family Poxviridae, subfamily Chordopoxvirinae.[1] The eight genera of vertebrate poxviruses are *Orthopoxvirus, Parapoxvirus, Avipoxvirus,*

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Capripoxvirus, Leporipoxvirus, Suipoxvirus, Molluscipoxvirus, and *Yatapoxvirus.* Only species of *Orthopoxvirus, Parapoxvirus, Molluscipoxvirus,* and *Yatapoxvirus* are known to infect humans. The last three are discussed in Chapter 133. Orthopoxvirus virions are large and brick shaped (as are the virions of yatapoxvirus and molluscipoxvirus). Poxvirus virions range in length from 220 to 450 nm and in width and depth from 140 to 260 nm.[11,12] The electron microscopic appearance of virions varies with sample preparation.[3] On cryoelectron microscopy of unstained, unfixed vitrified specimens, vaccinia and other orthopoxviruses appear as smooth, rounded rectangles; a uniform core is surrounded by a 30-nm membrane. In conventional thin sections, the core appears dumbbell shaped and is surrounded by a complex series of membranes. On negative stain, most virions have short surface tubules 10 nm in diameter and are referred to as M (mulberry) forms; a few virions, slightly larger and electron dense, appear to have a thick (20- to 25-nm) membrane or capsule (C form) (Fig. 132.1). Poxvirus particles contain about half of the approximately 200 potential virus genome-encoded proteins; virions are composed of structural proteins and enzymes including a virtually complete RNA polymerase system for primary transcription of viral genes.[3] The genome, which is within a nucleoprotein complex (nucleosome) inside the core, consists of a single linear molecule of double-stranded DNA that is composed, depending on the strain, of approximately 130 to 375 kb pairs of DNA and is covalently closed at each end; the ends are hairpin-like telomeres. Complete genome DNA sequences have been reported for several different species of the orthopoxviruses described in this chapter; GenBank entries are compiled at a dedicated website (https://www.viprbrc.org/brc/home.spg?decorator=pox).

During virus replication,[3,13,14] virion morphogenesis begins in the cytoplasm in areas known as cytoplasmic viral factories. Thin-section electron microscopic observations of cells early after infection show crescent-shaped membrane structures that progress to ovoid or circular structures, termed *immature virions,* which enclose a dense nucleoprotein complex. Primary transcription precedes the production of the crescents (cup shaped in three dimensions) and the immature virions. The bilayer surface membrane of the immature virion differentiates, with one layer becoming the outer membrane and the other becoming the core membrane, forming an intracellular mature virion (MV). A small portion of MVs may be further processed to acquire a bilayer envelope of Golgi intermediate compartment membrane that contains specific viral proteins. The intracellular enveloped virion (IEV) then moves along cellular microtubules to the cell surface, where actin polymerizes behind the IEV. IEVs exit the cell through distinctive microvilli by fusing the outermost lipoprotein layer with the plasma membrane, releasing the MV within the inner lipoprotein layer. The released particle is the extracellular enveloped virion (EEV); enveloped virions (EVs) that stay attached to the outer surface of the cell are cell-associated enveloped virions (CEVs). MVs and EVs are mature infectious particles, each with distinct surface antigenic properties. Enveloped and nonenveloped forms are believed to have different attachment sites on the cell[3]; however, the common result is fusion of the MV membrane with the plasma membrane, followed by entry and uncoating of the particle, release of viral contents into the cell, and initiation of virus-controlled transcription of early-, intermediate-, and late-class proteins.[3] Reviews of fusion and entry processes are available.[5,15,16] Secretion or expression of viral proteins modulates the host immune response or intracellular signaling to permit

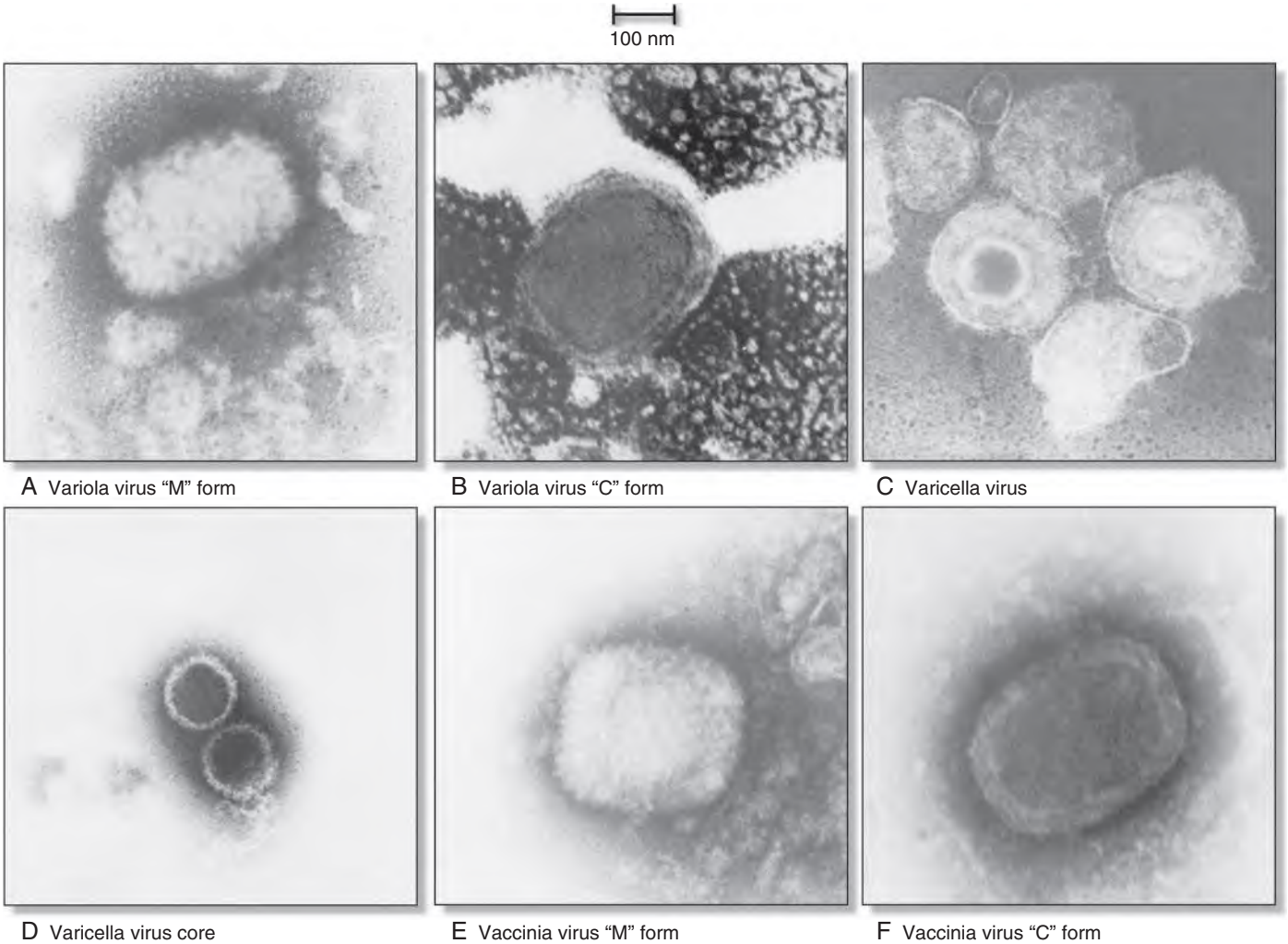

FIG. 132.1 (A–F) Electron micrographs of variola, varicella, and vaccinia virions. *(From Centers for Disease Control and Prevention [CDC] Public Health Information Library.* http://phil.cdc.gov/phil/default.asp. *Image ID 5 2426. Electron micrograph taken in 1975 by Dr. James Nakano, CDC.)*

the subsequent assembly, morphogenesis, release, and infectivity of progeny virions.

The dissemination of naturally released virions (EVs) is an important aspect of pathogenesis, and genes have been identified (e.g., *B5R*) that code for proteins necessary for the proper development of EEVs and expression of virulence. Host cell factors are also involved in EEV dissemination.[13,14,17]

PATHOGENESIS

Poxvirus infections evolve either as a localized, fairly benign skin infection or as a systemic infection. Systemic infection results in viral dissemination, with the formation of generalized skin lesions, and usually involves some degree of morbidity and mortality. The pathogenesis of human systemic orthopoxvirus infections is largely extrapolated and modeled from studies of the pathogenesis of the related orthopoxvirus infections in animals: mousepox (ectromelia) in mice, rabbitpox in rabbits, and monkeypox in nonhuman primates and prairie dogs.[18–23] In addition, extensive literature exists on animal models used to examine the role of virally encoded proteins predicted to be involved in viral pathogenesis or used to examine the effect of therapeutic methods or both.[24–27] The general paradigm for systemic orthopoxvirus pathogenesis is that the virus enters the host through a respiratory route, a mucosal surface, or a break in the skin and replicates locally. The virus then spreads through local lymphatics, causing a primary viremia, and subsequently spreads to the reticuloendothelial system. Replication in these organs results in the secondary viremia, usually associated with fever. The virus then ultimately seeds skin, causing a characteristic pock rash.

The host immune response probably involves all arms of the immune system. Complement, interferon, natural killer cells, and inflammatory cells are implicated in the early innate response; poxvirus-specific antibodies (including neutralizing antibodies) are subsequent components of the humoral response, and poxvirus-specific cytotoxic lymphocytes are involved in cellular clearance of infection.[28–35] Within their large genomes, poxviruses encode a number of proteins predicted to modulate the host's immune response, which affect both viral survival and disease pathogenesis. Various poxvirus-encoded proteins are predicted to bind and interfere with the function of host cytokines, chemokines, interferon, and complement. Other poxvirus-encoded proteins may interfere with apoptosis. Articles on immunomodulatory properties of poxviruses review specific properties of these virally encoded gene products.[5,36–38]

VACCINIA (SMALLPOX VACCINE)

Vaccinia is an orthopoxvirus and is the most studied of all poxviruses. It continues to be useful as a tool in medical research and is the focus of renewed interest as a vector for recombinant vaccines.[8] As such, it can pose an infection risk to laboratory personnel, and multiple exposures and infections have been documented in laboratory workers.[39] At some point, vaccinia replaced cowpox and horsepox[67] as the smallpox "vaccine." Genotypic analysis suggests that vaccine strains of vaccinia virus are most closely related to cowpox viruses from continental Europe and horsepox.[40] A single strain of the virus, derived from the New York City Board of Health (NYCBOH) strain, is used for smallpox vaccine in the United States. Other strains have been used outside the United States.

Smallpox vaccine is administered with a special bifurcated needle designed to hold a small standardized inoculum of a live virus suspension between its prongs. The skin over the deltoid or triceps is pierced multiple times with the needle with enough vigor to allow a trace of blood to appear after several seconds. Within 2 to 5 days of inoculation, a papule forms at the vaccination site. This papule evolves into a vesicle and then a pustule, reaching its maximum size (about 1 cm in diameter) by 8 to 10 days after vaccination, after which it dries to a scab, which usually separates by day 14 to 21.[41,42] An areola may encircle the site, and umbilication may occur as the lesion evolves. Low-grade fever is sometimes observed in children but rarely in adults. Regional lymphadenopathy may also occur. A scar at the inoculation site often provides lifelong evidence of successful vaccination, although the presence of a scar may not guarantee a history of successful smallpox vaccination because it may have resulted from bacterial superinfection or vaccination with the Calmette-Guérin bacillus.

Immunity Resulting From Vaccination

Vaccinia immunization is cross-protective against other orthopoxvirus infections including variola. Preexposure vaccine efficacy is estimated to be 100% for 1 to 3 years after vaccination,[43] and reports from the smallpox eradication efforts noted that "smallpox rarely occurs during the 4 or 5 years after successful vaccination in infancy."[44] Complete protection against smallpox after vaccination is not lifelong, although data suggest that substantial protection may persist for 15 to 20 years.[44–46] Experiences with monkeypox in the United States in 2003 suggest that immunity is not completely protective against systemic orthopoxvirus disease acquisition for more than 20 years after vaccination.[47,48] Protection against death from the disease may persist even longer than protection against disease.[49,50]

Likely because of the relatively long asymptomatic incubation period of systemic orthopoxvirus diseases (10–14 days), vaccinia is also effective as a postexposure prophylaxis when given to contacts of patients with smallpox. Vaccination should be performed as soon as possible after exposure; interpretation of data from the eradication program suggests that vaccination may not be as effective if given more than 3 days after the exposure.[51–54] Effectiveness was greater for individuals vaccinated previously.

Despite more recent advances, the correlates of immunity against smallpox are poorly understood. Humoral responses including neutralizing antibody correlate with protection in both animal[55] and human[56–58] studies. Cell-mediated and T-cell responses are also believed to be critical for successful vaccination.[59] T-cell responses have been documented up to 35 years after vaccination.[60–62] Neutralizing antibody responses are also long-lived.[61,63,64]

Complications Resulting From Vaccination

Of all vaccines used today, the smallpox vaccine has one of the highest rates of adverse events.[41,65–72] Major complications include progressive vaccinia, eczema vaccinatum, generalized vaccinia, accidental infection, postvaccinial encephalitis, and myopericarditis.

Progressive vaccinia, previously termed *vaccinia necrosum* or *vaccinia gangrenosum,* is a rare, often fatal vaccine complication in persons with severe deficiencies of cellular immunity.[65] In 1 year (1968) in the United States, five cases among 6 million primary vaccinations and six cases among 8.6 million revaccinations were found.[70] Four of these 11 patients died. Progressive vaccinia is characterized by progressive, often painless, growth and spread of the vaccine virus beyond the inoculation site, often leading to necrosis, sometimes with metastases to other body sites.[73] This diagnosis should be considered if the vaccination site lesion continues to progress and expand without apparent healing more than 15 days after vaccination.[74] Initially, limited or no inflammation is present at the site, and histopathologic examination shows an absence of inflammatory cells.[75] Management of progressive vaccinia has historically included aggressive therapy with vaccinia immune globulin (VIG), methisazone, débridement, and whole-blood transfusions from previously vaccinated individuals. The last-mentioned procedure, designed to bolster cell-mediated immunity, often resulted in a graft-versus-host reaction.[73] Methisazone is now regarded as ineffective therapy. Supportive measures and attention to prevention of secondary bacterial infections are beneficial, and newer therapeutic agents such as tecovirimat (TPOXX or ST-246) (discussed further in Chapter 48), cidofovir, and brincidofovir (CMX-001), may also have a role, although their efficacy is unproven (discussed further in "Therapy" and in Chapters 46 and 48).[41,66] VIG is available from the Centers for Disease Control and Prevention (CDC) as an intravenous formulation. In 2009, a severe case of progressive vaccinia developed in a member of the US Armed Forces after vaccination against smallpox and a subsequent diagnosis of acute myelogenous leukemia (subtype M0) and initiation of chemotherapy. The patient recovered after intensive treatment including multiple doses of vaccinia immune globulin intravenous (VIGIV), oral and topical tecovirimat, brincidofovir, and imiquimod.[67,68]

Eczema vaccinatum can occur in people with a history of atopic dermatitis (eczema) regardless of disease severity or activity. This complication is the clinical result of local spread or dissemination from the primary vaccination site in such persons or the result of inadvertent

contact of another's unscabbed vaccination site with a susceptible atopic individual's skin.[76,77] The clinical presentation is a localized or generalized papular, vesicular, or pustular rash anywhere on the body or localized to previous eczematous lesions. Systemic illness with fever, malaise, and lymphadenopathy may occur. In the 1968 national survey, 66 cases (no deaths) among 14.5 million vaccinations (4.6 cases per 1 million) and 60 cases (1 death) among several million contacts were found. Treatment of eczema vaccinatum includes administration of VIGIV (6000 U/kg, with repeat doses or higher doses or both depending on severity of symptoms and response to treatment),[43] hemodynamic support with fluid replacement and electrolyte monitoring, and skin care.[41] In one study, early VIG administration reduced the mortality rate from 30% to 40% to 7%.[78] In 2007 a severe case of eczema vaccinatum was successfully treated with a combination of multiple doses of VIGIV, a single dose of cidofovir, a multiple-day course of oral tecovirimat, intensive skin care management, and skin grafting.[79]

Generalized vaccinia is a nonspecific term that is used to describe a vesicular rash that develops after vaccination. True generalized vaccinia is believed to represent the end product of viremic spread of virus. However, documentation of virus in suspected generalized vaccinia vesicular rash lesions has been extremely rare, leading to the proposal of a new dermatologic manifestation termed *postvaccinial nonviral pustulosis* to explain these findings.[80,81] No predisposing factors have been identified. Treatment is generally not necessary because the generalized rash is self-limited. The lesions evolve and resolve more quickly than the primary vaccination site, presumably because of a developing immune response to the virus. This complication is estimated to occur in approximately 242 of every 1 million primary vaccinations.[41] In general, this complication of vaccination does not necessitate the administration of VIG unless it is severe and the patient is systemically ill or the patient has an underlying immunocompromising condition. Treatment with nonsteroidal antiinflammatory drugs or oral antipruritics may provide symptomatic relief.[41]

Postvaccination encephalomyelitis (PVEM) is a rare but serious complication that usually occurs only in primary vaccinees. The frequency of its occurrence differs widely from country to country and with the strain of vaccinia virus used in the vaccine. In the survey conducted in the United States in 1968, the frequency was 2.9 to 12.3 per 1 million first-time vaccinees.[41] The incidence of PVEM was lower with the NYCBOH vaccinia virus strain than with the strain used in other countries.[82] No predisposing factors are known, although host factors are believed to be important; the pathophysiology is not well understood. Cases have variably displayed clinical and diagnostic features suggestive of a postimmunization demyelinating encephalomyelitis/acute demyelinating encephalomyelitis or direct viral invasion of the nervous system.[83–85] This postvaccination reaction typically occurs 11 to 15 days after vaccination. Symptoms of PVEM include fever, headache, vomiting, confusion, delirium, disorientation, restlessness, drowsiness or lethargy, seizures, and coma. The cerebrospinal fluid can have an elevated pressure but generally has a normal cell count and chemistry profile.[86–88]

Children younger than 2 years can also develop a rare postvaccination encephalopathy (PVE) similar to PVEM. Acute onset of PVE occurs earlier in the postvaccination period (6–10 days after vaccination), involves the same symptoms as PVEM, and may include hemiplegia and aphasia.[4,41]

The diagnosis of PVE or PVEM is one of exclusion because no specific tests are available to confirm the diagnosis of this complication, and many other infectious and toxic etiologies can result in a similar clinical picture. More recent cases have shown the presence of orthopoxvirus-reactive antibodies in cerebrospinal fluid.[89,90] The role of antiviral medications is unclear; VIG, although effective prophylactically, has not shown clear benefit after symptom onset. A few cases have responded to some combination of intravenous immune globulin, steroids, and VIG.[89] A review of the pathogenesis of acute demyelinating encephalomyelitis and suggested therapies is available (also see Chapter 89).[91]

Accidental infection occurs when virus from the vaccination site is transferred to another site or to another person through intimate skin contact. It usually occurs in individuals receiving primary vaccination rather than a repeat vaccination. Accidental self-inoculation, which most commonly occurs on the face, mouth, lips, or genitalia, is usually not serious and requires no specific treatment. Inoculation of the conjunctiva, cornea, or eyelid is more serious and can be sight threatening if not evaluated and treated appropriately. In the years 1963–68, ocular vaccinia was observed in 348 people (259 vaccinees and 66 contacts). Of these, 22 had evidence of corneal involvement, and 11 had permanent defects.[92] No controlled trials of therapeutics exist; current topical optic antivirals (trifluorothymidine, vidarabine)[93] have in vitro activity against vaccinia, and their off-label use for this purpose has been recommended by some ophthalmologists (see Chapter 113).[41]

Cardiac adverse events are rare and had not been reported before 2003 in any person vaccinated with the NYCBOH strain. Myocarditis had been reported after vaccination with the vaccine strains used in Europe and Australia,[94,95] and in the US military population, myopericarditis was documented in 18 of 230,734 primary vaccinees immunized with the NYCBOH strain in 2002–03.[96] Clinical studies suggest that myopericarditis may occur in 1 in 175 adults who receive primary vaccination against smallpox.[97] However, few documented cases of dilated cardiomyopathy exist, and most cases appear to be mild and self-limited.[98,99] Arrhythmias and myocardial ischemia have also been described, but the association with vaccination is not as clear.[100–104]

Vaccination programs implemented in 2002–03 designed to help civilian public health preparedness and military preparedness for the possible use of smallpox as a weapon of bioterrorism documented lower adverse event rates than previously seen, in part because of stringent criteria and education programs to screen persons at risk for complications.[100,105,106] Nonetheless, instances of generalized rash, which may arise 10 to 14 days after vaccination, continued to be reported. Diagnosing true generalized vaccinia, which represents virus presumably spread hematogenously, on a clinical basis alone is often difficult, as it can be confused with a form of erythema multiforme or erythematous urticaria eruptions that may be immunologically mediated. Laboratory identification of virus within the disseminated rash may differentiate these conditions. Studies of recent vaccination efforts have also identified focal and generalized folliculitis associated with vaccination.[107]

The recommendations of the Advisory Committee on Immunization Practices on vaccinia vaccination are available at https://www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/smallpox.html. Additional clinical guidance for the use of smallpox vaccines in a smallpox emergency have been published.[108] The Advisory Committee on Immunization Practices recommends vaccination as a safeguard for laboratory and health care workers who are at high risk for orthopoxvirus infection.[109] In the United States the CDC Drug Service provides the vaccine after CDC approval of a formal request for this purpose by the administering physician. Vaccinia immunoglobulin is available to treat possible postvaccination complications, which can be severe.[110] In the United States the use of a cell culture–grown clonal derivative of the NYCBOH/Dryvax (Wyeth Pharmaceuticals, Collegeville, PA) smallpox vaccine has replaced use of Dryvax. Studies and development of this new vaccine, ACAM2000, have been previously reviewed.[97,111]

Vaccinia Virus as a Zoonosis

Vaccinia virus infections are not generally regarded as naturally occurring, although vaccinee-to-cattle and cattle-to-human transmissions occurred on farms during the smallpox eradication campaign. Sporadic outbreaks of infection caused by the vaccinia virus subspecies buffalopox virus, which involve transmission between milking buffalo, cattle, and people, have been reported mainly in India but also in Egypt, Bangladesh, Pakistan, and Indonesia. Vaccinia-like lesions have been observed on the animals' teats and the milkers' hands. Biologic data and limited DNA analyses of isolates from an outbreak in India in 1985 suggest that buffalopox virus may be derived from vaccinia virus strains transmitted from humans to livestock during the smallpox vaccination era.[112–114] A vaccinia virus possibly related to the vaccine strain used during smallpox eradication in Brazil was found in cattle and farm worker handlers in rural Rio de Janeiro.[115,116] Subsequent reports and surveillance efforts indicate that these vaccinia virus infections are ongoing and that at least two clades or subgroups of vaccinia are circulating in Brazil, likely representing low-level endemic disease.[117–119] Vaccinia virus infections of cattle and farm workers have also been identified in

FIG. 132.2 Smallpox lesions on skin of the trunk. *(From Centers for Disease Control and Prevention [CDC] Public Health Information Library. http://phil.cdc.gov/phil/default.asp. Image ID 5 284. Photograph taken in 1973 by James Hicks, CDC.)*

FIG. 132.3 Smallpox lesions on palms. This child was infected with the smallpox virus and on day 8 of the rash shows the typical lesions on his palms. *(From Centers for Disease Control and Prevention [CDC] Public Health Information Library. http://phil.cdc.gov/phil/default.asp. Image ID 5 3303. Photograph taken in 1972 by Dr. Paul B. Dean, CDC.)*

Colombia.[120] The ecologic factors that support the persistence of vaccinia in this region of the world remain unknown and are a topic of study.[121,122] In addition, at least two instances of human infection with a vaccinia-vector recombinant rabies virus vaccine in a bait dispersed to control rabies in wildlife have been reported; the bait was retrieved by a pet dog in both cases.[123,124]

VARIOLA (SMALLPOX)

In 1980 the WHO General Assembly declared that smallpox had been eradicated; destruction of the remaining virus stocks, initially scheduled for 1999, has been delayed in an effort to permit research for improved preparedness in the event smallpox recurs as the result of the malevolent use of variola virus. The virus has a strict human host range and no animal reservoir. Variola major strains produced a disease with a severe prodrome, fever, and prostration. The virus was most often transmitted between humans via large-droplet respiratory particles inhaled by susceptible persons who had prolonged close face-to-face contact with an infectious person; it was spread less commonly via aerosol or direct contact with the rash lesion or sloughed crust material from the scab.[82,125] A toxemia or other form of systemic shock led to case-fatality rates of up to 30%. Secondary attack rates among unvaccinated contacts within households ranged from 30% to 80%. Variola minor strains (alastrim, amass, or kaffir viruses) produced less severe infection and case-fatality rates of less than 1%, although secondary attack rates among unvaccinated contacts within households also ranged from 30% to 80%. The last naturally occurring smallpox case was in Somalia in October 1977, although a fatal laboratory-associated infection with variola major virus occurred at the University of Birmingham, England, in August 1978.[82]

Naturally acquired variola virus infection caused a systemic febrile rash illness. For ordinary smallpox, the most common clinical presentation, after an asymptomatic incubation period of 10 to 14 days (range, 7–17 days), was fever, with the temperature quickly rising to about 103°F, sometimes with dermal petechiae. Associated constitutional symptoms included backache, headache, vomiting, and prostration. Within 1 or 2 days after incubation, a systemic rash appeared that was characteristically centrifugally distributed (i.e., lesions were present in greater numbers on the oral mucosa, face, and extremities than on the trunk). Lesions were commonly manifested on the palms and soles (Figs. 132.2 and 132.3). Initially, the rash lesions appeared macular, then papular, enlarging and progressing to a vesicle by days 4 to 5 and a pustule by day 7; lesions were encrusted and scabby by day 14 and sloughed off (Fig. 132.4). Skin lesions were deep-seated and in the same stage of development in any one area of the body. Milder and more severe forms of the rash were also documented. Less severe manifestations (modified smallpox or variola sine eruptione) occurred in some vaccinated individuals, whereas hemorrhagic or flat-pox types of smallpox

FIG. 132.4 Smallpox lesions at day 17 of rash on a 5-year-old convalescing Indonesian child. *(From Centers for Disease Control and Prevention [CDC] Public Health Information Library. http://phil.cdc.gov/phil/default.asp. Image ID 5 2041. Photograph taken in 1963 by J.D. Millar, CDC.)*

are thought to have developed as a result of impaired immune response of patients.

Variola major smallpox was differentiated into the following four main clinical types:

1. Ordinary smallpox (90% of cases) produced viremia, fever, prostration, and rash; mortality rates were generally proportionate to the extent of rash. With the WHO classification, mortality rates ranged from less than 10% for "ordinary discrete" smallpox to 50% to 75% for the rarer "ordinary confluent" presentation.
2. (Vaccine)-modified smallpox (5% of cases) produced a mild prodrome with few skin lesions in previously vaccinated people and a mortality rate well under 10%.
3. Flat smallpox (5% of cases) produced slowly developing focal lesions with generalized infection and an approximate 50% fatality rate.

4. Hemorrhagic smallpox (<1% of cases) induced bleeding into the skin and the mucous membranes and was invariably fatal within a week of onset.

A discrete type of the ordinary form, with a typical febrile prodrome and rash, resulted from alastrim variola minor infection.[82] The WHO established a classification system for smallpox case types based on disease presentation and rash burden. The hemorrhagic and flat types have already been briefly described. The ordinary type was subdivided into three categories on the basis of the extent of rash on the face and the body. In the ordinary confluent category, no area of skin was visible between vesiculopustular rash lesions on the trunk or the face. Patches of normal skin were visible between rash lesions on the trunk in ordinary semiconfluent disease and on the face in ordinary discrete disease. (Vaccine)-modified disease arose with sparse numbers of lesions. Infection conferred lifelong immunity.[82,126]

Before its eradication, smallpox as a clinical entity was relatively easy to recognize, but other exanthematous illnesses were mistaken for this disease.[125–127] For example, the rash of severe chickenpox, caused by varicella-zoster virus, was often misdiagnosed as that of smallpox. However, chickenpox produces a centripetally distributed rash and rarely appears on the palms and soles. In addition, in the case of chickenpox, prodromal fever and systemic manifestations are mild, if they occur at all; the lesions are superficial in nature; and lesions in different developmental stages may be present in the same area of the body. Other diseases confused with vesicular-stage smallpox included monkeypox, generalized vaccinia, disseminated herpes zoster, disseminated herpes simplex virus infection, drug reactions (eruptions), erythema multiforme, enteroviral infections, scabies, insect bites, impetigo, and molluscum contagiosum. Diseases confused with hemorrhagic smallpox included acute leukemia, meningococcemia, and idiopathic thrombocytopenic purpura. The CDC, in collaboration with numerous professional organizations, has developed an algorithm for evaluation of patients with smallpox. The algorithm assists in differential diagnoses of the vesiculopustular stage of rash. The algorithm and additional laboratory testing information are available at https://www.cdc.gov/smallpox/clinicians/diagnosis-evaluation.html. The differential diagnosis of febrile vesicular pustular rash illnesses is presented in Table 132.1.

TABLE 132.1 Differential Diagnosis of Febrile Vesicular Pustular Rash Illnesses That May Be Confused With Smallpox

DISEASE	CLUES
Varicella	Most common in children younger than 10 years; children do not usually have a viral prodrome
Disseminated herpes zoster	Immunocompromised or elderly persons; rash looks like varicella, usually begins or erupts in dermatomal pattern
Impetigo *(Streptococcus pyogenes, Staphylococcus aureus)*	Honey-colored crusted plaques with bullae are classic but may begin as vesicles
Drug eruptions	Exposure to medications
Erythema multiforme minor	Target or bull's-eye lesions; often follows systemic viral infections such as herpes simplex virus; may include palms and soles
Erythema multiforme (including Stevens-Johnson syndrome)	Involves conjunctivae and mucous membranes
Enteroviral infections (especially hand-foot-and-mouth disease)	Seasonal—summer and fall
Disseminated herpes simplex virus	Similar to varicella
Scabies and insect bites	Pruritus; patient not febrile
Molluscum contagiosum	May disseminate in immunosuppressed individuals
Generalized vaccinia	History of vaccination with smallpox vaccine or contact with vaccinated individual
Monkeypox	Travel to endemic area; animal exposure

Modified from Centers for Disease Control and Prevention. Evaluating patients for smallpox: acute generalized vesicular or pustular rash illness protocol. https://www.cdc.gov/smallpox/clinicians/algorithm-protocol.html.

MONKEYPOX

Monkeypox virus was so named because it was first detected in captive Asiatic monkeys; however, the virus has been found naturally only in Africa (although it emerged in the United States as a result of global commerce), and evidence points to rodents as important reservoir hosts. Reviews of human monkeypox infection are available.[128–130]

Monkeypox was first recognized by Von Magnus in Copenhagen in 1958 as an exanthem of primates in captivity. The disease was later seen in other captive animals including primates in zoos and animal import centers. Particular attention was focused on it in 1970 when smallpox surveillance activities in Africa revealed cases of human monkeypox, clinically indistinguishable from smallpox, particularly in Zaire (now the Democratic Republic of the Congo [DRC]). Serosurveys and virologic investigations in the 1980s in the DRC by the WHO indicated that monkeys are sporadically infected, as are humans; three-fourths of cases, mainly in children younger than 15 years, resulted from animal contact; vaccinia vaccination has about 85% protective efficacy; monkeypox virus probably has a broad host range including squirrels (*Funisciurus* spp. and *Heliosciurus* spp.); and human monkeypox has a secondary attack rate of 9% among unvaccinated contacts within households (i.e., it is much less transmissible than smallpox). Since 1970 the disease has been seen in the DRC, Liberia, Ivory Coast, Sierra Leone, Nigeria, Benin, Cameroon, Gabon, Central African Republic, and South Sudan; most cases have been in the DRC, which in 1980 had a population of about 30 million (338 cases were discovered prospectively during WHO-intensified monkeypox surveillance in Zaire in 1981–86). Human monkeypox has continued to be reported from the DRC, mainly in children younger than 15 years. On the basis of reported monkeypox onset dates in a largely retrospective study complicated by a concurrent outbreak of chickenpox, about 250 serosubstantiated cases of monkeypox occurred among 0.5 million people in 78 villages, from February 1996 to October 1997. About three-fourths of the cases appeared to result from human-to-human transmission; however, the secondary attack rate of 8% among unvaccinated contacts within households appeared to be about the same as in the 1981–86 surveillance.[128,131]

Sporadic outbreaks continue to occur and cause concern,[128,132] but the most detailed clinical, epidemiologic, and ecologic information about virology laboratory–confirmed disease in Africa was obtained before 1988. Initial animal surveys in Zaire detected monkeypox-specific antibodies in 85 of 347 squirrels (25%) sampled but from none of 233 terrestrial rodents. Monkeypox-specific antibody has been detected in very few monkeys, which, similar to humans, are probably only occasional hosts.[133] Subsequent work[131] in the DRC found evidence of orthopoxvirus seroreactivity in some small terrestrial mammals tested including Gambian rats *(Cricetomys emini)* and elephant shrews *(Petrodromus tetradactylus)*. Studies in the 1980s that used direct virus sampling of trapped animals revealed virus in only one *Funisciurus* species.

In 2003 monkeypox infection of humans was identified in the United States as a result of exposure to ill prairie dogs, probably infected after exposure to infected West African small mammals imported as exotic pets.[134] From that work, a Gambian rat *(Cricetomys gambianus)*, rope squirrel (*Funisciurus* sp.), and dormouse (*Graphiurus* sp.) from the affected African shipment of exotic species originating in Ghana and implicated in the US monkeypox outbreak were found to be infected with monkeypox with viral isolation and nucleic acid detection (polymerase chain reaction).[134]

Pathogenesis

The pathogenesis of human monkeypox is surmised to be similar to that of smallpox: an acute febrile exanthem with an incubation period of about 12 days. During the incubation period, the virus is distributed initially to internal organs and then to the skin.[82,129] The main differences are a greater degree of lymphadenopathy and a lower capacity for human

case-to-case spread. The major concerns are the source of infection and the mode of transmission.

Clinical Manifestations

In general, the clinical features of disease as seen in Central Africa are those of a classic or modified case of smallpox. The most obvious difference is the pronounced lymphadenopathy, which involves the submandibular, cervical, and sublingual regions.

Most cases occur in unvaccinated children. In the DRC in 1981–86, 291 cases (86%) occurred in children younger than 10 years, and only 12 of them (4%) had vaccination scars. The illness lasts 2 to 4 weeks. Of 292 unvaccinated patients, 22 (7.5%) had a mild illness with fewer than 25 skin lesions and were not incapacitated; 55 (19%) had 25 to 99 lesions, were incapable of most physical activity, and needed nursing; and 218 (75%) had more than 100 lesions, were totally incapacitated, and needed intensive nursing. Complications occurred in about 40% of patients; the most common were bacterial skin infections (16%), respiratory (12%) and gastrointestinal (5%) disorders, and keratitis (3.8%). The overall mortality rate was approximately 10%; however, all the deaths occurred in unvaccinated children, in whom the group mortality rate is about 15%.[129] In the United States, disease in the 2003 outbreak appears to have been milder, and only 3 of the 37 laboratory-confirmed cases had complications or serious disease: keratitis, encephalopathy (with diffuse cortical, thalamic, and brainstem edema; meningeal enhancement; and left thalamic and right parietal signal abnormality on magnetic resonance imaging),[134] and upper respiratory tract lymphadenitis with dysphagia and airway compromise.[135] No deaths were observed. Although routes of exposure and host factors may have been significantly different in the African and US outbreaks, evidence of differences between DRC/Congo Basin and US/West African viral strains/clades is also evident.[136–138] Additional animal and human studies are beginning to define some of the viral genetic differences that may contribute to the differences in pathogenicity and apparent interhuman transmissibility of the two viral clades.[139,140] The skin lesions of monkeypox are illustrated in Fig. 132.5.

Diagnosis

Until 2003, human monkeypox had not been detected outside Africa. Clinical diagnosis may present a problem because fewer physicians now have experience with smallpox, which human monkeypox closely resembles. In vaccinated individuals, the rash may be more pleomorphic and not in a uniform stage of development.[129] Access to a virology laboratory should permit detection of the virus with electron microscopy and molecular methods, and this detection provides a diagnosis. In some circumstances, distinguishing between monkeypox and tanapox may be important. In the past, differentiation between monkeypox and variola was essential and could be done with examination of the pock appearance (hemorrhagic or not) and presence of pocks produced on chorioallantoic membrane at 39°C, a temperature that inhibits smallpox. Currently an array of nucleic acid diagnostic techniques permits the speciation of these viruses.[141–146]

The close serologic relationships among orthopoxviruses make detection of monkeypox-specific antigens difficult, but methods are available and being refined that may be of value, particularly for epidemiologic studies of monkeypox virus in its natural reservoirs.[11,133] An immunoglobulin M capture assay was shown to be sensitive and specific in identification of monkeypox cases 4 to 56 days after rash onset; this assay may have similar use in the identification of human smallpox cases.[147]

FIG. 132.5 Monkeypox skin lesions.

Epidemiology and Control

Management of individual cases has been supportive, with case-to-case spread reduced with isolation and, if available, the use of smallpox vaccine for contacts. Potential use of newer therapeutics is discussed at the end of this chapter. Human cases in Africa occur in villages in the rain forests, where a variety of animals are captured for food. Infection of children may be explained by their playing or working with carcasses. The results of the comprehensive surveys carried out in the 1980s indicated that infected individuals were principally unvaccinated children and that case-to-case spread was unusual. Control measures are based on interposing a buffer zone of cleared land between the arboreal reservoir and cultivated land, the development of animal husbandry as a source of meat, and education in the handling of wildlife, with emphasis on any trapping done by previously vaccinated individuals; continued vaccination was not thought necessary.[129,133]

Only occasional human cases were reported from Central Africa after cessation of routine surveillance activities following smallpox eradication, but a resurgence occurred in 1996–97 that has not yet been fully explained. Increased political unrest leads to population displacement and breakdown of routine control measures, and the levels of vaccine-induced immunity decline with time. A potentially serious finding that requires clarification is the observation that human case-to-case transmission appears to have occurred more frequently in 1996–97 than earlier.[132] A study of an outbreak in the Republic of the Congo also showed that the virus was transmitted quite efficiently between successive generations of human transmission associated with a hospital compound; six generations of uninterrupted interhuman transmission were identified and supported with laboratory studies.[148] Human monkeypox continues to be reported in the Congo Basin, and mildly symptomatic monkeypox or other orthopoxvirus infections may coexist in the area.[149–151] Congo Basin clade monkeypox was reported in Cameroon, Central Africa Republic, and Republic of Congo in 2017.[152] A sizable outbreak of monkeypox was reported in Nigeria in 2017 with multiple states affected. As of September 2018, more than 100 confirmed cases of monkeypox have been reported from Nigeria.[153] Comparison of the genomes of smallpox virus and monkeypox virus strains isolated up to 1986 suggested that they have evolved discretely,[154] and the results of complete genome analysis[155] confirm this observation. Laboratory workers studying monkeypox virus should be vaccinated with vaccinia and handle the virus in certified biosafety cabinets. Biosafety Level 2 (BSL-2) containment, according to the *Biosafety in Microbiological and Biomedical Laboratories* definition, should be the minimum containment used. BSL-3 laboratory practices provide additional biosafety protection. As mentioned previously, additional select agent requirements apply to use of the Congo Basin clade of this virus.

COWPOX

Cowpox sometimes occurs as a rare occupational infection of humans and can be acquired by contact with infected cows; more often, other animals (e.g., infected rats, pet cats, and zoo and circus elephants) have been sources of the disease. More recently, two cases of laboratory-acquired human infection with cowpox virus were documented.[156,157] Phylogenies show that cowpox virus is a diverse species and is geographically restricted; it has been isolated from humans and various animals in Europe and adjoining regions of Asia.[158–160] A serosurvey of wild animals in Great Britain found orthopoxvirus antibodies in a portion of bank and field voles and wood mice that were collected, which is consistent with small rodents being reservoir hosts for cowpox virus.[161]

Clinical Manifestations

Most information is available from a detailed analysis of 54 human cases investigated in 1969–93.[159] Lesions are generally restricted to the hands and face, and most patients (72%) have only one lesion. Multiple lesions may be caused by multiple primary inoculations, by autoinoculation, and occasionally by lymphatic or viremic spread. Lesions in humans occur mainly on the fingers, with reddening and swelling; autoinoculation of other parts of the body may occur, and systemic severe infections have been reported, often in individuals with immunosuppressive conditions.[162] Skin lesions are initially similar to lesions of a primary vaccinia virus vaccination; the site becomes papular, and in 4 to 5 days a vesicle develops. The lesion passes through macular, papular, vesicular, and pustular stages before forming a hard black crust. The lesion is usually painful, and erythema and edema are common at the late vesicular and pustular stages. Usually, lymphadenitis, fever, and general malaise occur, often referred to as influenza-like. These features are usually severe in children; 16 of 54 patients (30%) were hospitalized. Most patients take 6 to 8 weeks to recover; for some patients, recovery takes more than 12 weeks. Scarring is usually permanent.

NEWLY DESCRIBED ORTHOPOXVIRUSES

Human infection with a novel orthopoxvirus was detected in the country of Georgia.[163] Two men developed poxlike lesions on their hands after tending to ill cows. Both eventually recovered fully with some scarring. Although cowpox was suspected, DNA sequencing revealed a novel virus that, although related to known Old World orthopoxviruses, had significant phylogenetic branching divergences. Epidemiologic investigations suggested that cows were likely to be incidental hosts of this novel virus, whereas the natural reservoir may be small mammals such as shrews and rodents.[163] Other orthopoxviruses or closely related viruses have been characterized as the cause of human infections. These infections include the localized rash lesions in a resident of Alaska (AK2015)[164] and the infection of a renal transplant patient on an immunosuppressive regimen with a progressively spreading rash illness (NY-v014).[164,165]

ORTHOPOXVIRUS LABORATORY DIAGNOSTICS

Electron microscopy of vesicle fluid or extracts of crusts is particularly valuable because it distinguishes between parapoxvirus, herpesvirus, and other presumptive orthopoxvirus infections. Of 24 cases of cowpox in which adequate material was available, electron microscopy was successful in 23. Similar sensitivity was achieved in the diagnosis of smallpox.[12] Many protein-based diagnostic approaches have also been used[5,11] and further developed.[166] Nucleic acid–based diagnostics[142–144] are now commonly used to diagnose orthopoxvirus infections and to speciate them. Within currently defined species, not all strains of cowpox are identical, and genomic analysis may show differences of epidemiologic value. As well, although the phylogenetic differences are not as great within the monkeypox virus and vaccinia virus species, diagnostic techniques have been developed to differentiate monkeypox virus clades and vaccinia strains.

Virus may be isolated on the chorioallantoic membrane, where the production of characteristic hemorrhagic pocks is diagnostic. A cytopathic effect occurs in many cell lines (Vero, MRC-5, RK13), and detection of A-type inclusions is usually diagnostic for human cowpox virus infections, as it would be if found in biopsy material.

THERAPY

An active area of research involves the development and evaluation of therapeutics for orthopoxvirus infections. VIGIV is prepared from serum from individuals who have received smallpox vaccination. It has been licensed by the US Food and Drug Administration and is available through the CDC (https://www.cdc.gov/smallpox/clinicians/vaccine-medical-management6.html). Although controlled clinical trials of the efficacy of VIGIV have not been carried out, it has been used in the treatment of eczema vaccinatum, progressive vaccinia, and generalized vaccinia.[41] It can be considered for use in ocular complications of vaccinia except for vaccinia keratitis, in which it may be harmful (see Chapter 113). No clear benefit has been shown for vaccinia immune globulin alone in treatment of smallpox, but it may be useful in the treatment of other orthopoxvirus infections (see later). Comprehensive reviews of the history and latest developments in poxvirus antivirals and therapeutics have been published.[5,93,167] In addition, such compounds are being tested on experimental models of variola-infected nonhuman primates[168] and on other animal models of orthopoxvirus infections.[25,26,169] The earliest drug compounds shown to have activity against orthopoxviruses including vaccinia were thiosemicarbazone derivatives. Initial case studies and case series suggested that they were effective in prophylaxis of smallpox and in treatment of progressive vaccinia and eczema vaccinatum.[170,171] However, subsequent double-blind controlled trials of smallpox prophylaxis revealed no benefit.[172]

A number of different classes of therapeutics have been tested for potential systemic or topical use against orthopoxviruses. These include compounds predicted to interfere with specific viral enzymes and cellular targets.[167] In vitro studies have usually focused on compounds currently licensed or under phase I or II study, although some novel compounds have also been studied. Promising candidates have been studied in small animal models (mouse, rabbit, and others) and in nonhuman primates. Because monkeypox and smallpox both cause human illness, with mortality rates that range from 10% to 40% in nonvaccinated individuals, models have been designed to evaluate drug efficacy in systemic lethal disease created by intranasal, aerosol, or (historically) intracerebral virus challenge. Intravenous challenges classically have been designed to evaluate the effect of drug on rash development or illness progression, although higher challenge doses of virus have been used for a lethal model.[173] The primary animal models currently used involve a challenge of aerosolized or intranasal virus, which results in pulmonary disease and lethality. Models of localized rash lesions have involved scarification of animal skin. Treatment models of keratitis involve scarification of corneal tissue. Work evaluating the potential use of antivirals for treatment of systemic complications of vaccination (progressive vaccinia and eczema vaccinatum) has used immunodeficient mouse populations.

The most studied compounds are inhibitors of DNA polymerase. Some nucleoside analogue compounds with activity against herpesviruses, most notably acyclovir and its derivatives, do not have activity against poxviruses. Other compounds with antiherpesvirus activity do show in vitro and in vivo activity against poxviruses, specifically 5-iodo-2′-deoxyuridine, adenine arabinoside, and trifluorothymidine.[174–177] Because of their systemic toxicity, these compounds have also been used topically for treatment of orthopoxvirus (and herpesvirus) ocular infections. Of the three compounds, trifluorothymidine appears to be most widely available. Many phosphonate-nucleoside analogues (e.g., cidofovir) have antiorthopoxvirus activity. In vitro, cidofovir has been shown to be active against the orthopoxviruses cowpox, vaccinia, monkeypox, and variola.[26,178,179] In vivo studies with cidofovir have shown it to successfully protect challenged animals when given prophylactically or early in the evolution of disease, often before the onset of overt symptoms. Cidofovir has known renal toxicity and is administered with hydration and probenecid. It has a long intracellular half-life but is not orally bioavailable; the alkoxyalkyl ester analogue of cidofovir, 1-*O*-hexadecyloxypropyl cidofovir (brincidofovir), is orally bioavailable and has been reported to have a protective effect in mice challenged with aerosolized cowpox, mice challenged with a lethal inoculum of mousepox virus, and rabbits challenged with a lethal inoculum of rabbitpox virus.[179–181] Brincidofovir has also been shown to be active against variola virus in tissue culture.[182] Other nucleoside analogues are also under study.[183,184]

Tecovirimat (ST-246 or TPOXX) is a novel orally bioavailable compound with antiorthopoxvirus activity, characterized to inhibit orthopoxvirus release. Genetic characterization of viral mutants resistant to tecovirimat indicate the viral target is the orthopoxvirus homologue of the vaccinia F13 protein, which is needed for wrapping of the virus before its release as an enveloped viral particle.[185] The drug has been shown to be active against multiple orthopoxvirus species, including monkeypox and variola virus.[186,187] Tecovirimat, used prophylactically before the onset of symptoms or therapeutically after the onset of symptoms, has been shown in a variety of small animal

models to prevent disease or to significantly mitigate illness severity and mortality.[188–190] As mentioned earlier in the section on vaccinia virus, it has been used, in combination with other therapeutic methods, in the successful treatment of severe cases of eczema vaccinatum and progressive vaccinia. Animal studies have shown the drug has synergistic benefit when combined with brincidofovir.[191] The drug was approved by the US Food and Drug Administration in July 2018 for the treatment of human smallpox disease. (Tecovirmat is discussed further in Chapter 48.)

Other antivirals tested against orthopoxviruses have predicted cellular targets. Ribavirin, an inosine monophosphate dehydrogenase inhibitor, shows in vitro activity against a number of orthopoxviruses[192] and has shown antiorthopoxvirus activity in animal models of vaccinia-induced keratitis[193] and mouse tail pock lesions.[194] Case reports of the use of ribavirin and VIG in the treatment of progressive vaccinia are available.[195] Initial therapy with ribavirin alone was ineffective at stemming new lesions; however, with the addition of VIG, new lesion development was stopped.

Additional studies of compounds targeting cellular kinases are of interest as another potential antiorthopoxvirus therapeutic strategy. Various cellular kinases (Abl, Src, and others) have been shown to be involved in the egress of virus, and blocking their function provides a different "antiviral" therapeutic mechanism of action.[196,197] Studies of an ErbB kinase inhibitor (CI-1033) showed benefit in treatment of vaccinia-infected mice; its benefit was augmented with the use of a monoclonal antibody that neutralizes MV viral particles. In vitro, CI-1033 appeared to inhibit the local release of virus from an infected cell, so as to impede the ability of the virus to subsequently infect other surrounding (uninfected) cells.[198]

Comprehensive summaries of antiorthopoxvirus therapeutic development are available,[5,167,17,199–202] and this is an area of active research. For example, a study evaluating a 19th century therapy for smallpox involving a botanical preparation of the carnivorous plant *Sarracenia purpurea* demonstrated antiorthopoxvirus activity against vaccinia, monkeypox, and variola viruses.[203]

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The complete reference list is available online at Expert Consult.

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133 Other Poxviruses That Infect Humans: Parapoxviruses (Including Orf Virus), Molluscum Contagiosum, and Yatapoxviruses

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SHORT VIEW SUMMARY

Definition
- Parapoxviruses, molluscum contagiosum, and yatapoxviruses are among the nonorthopoxvirus infections of humans.

Epidemiology
- Parapoxvirus infections most commonly occur in individuals with occupational exposures to infected sheep, cattle, or goats.
- Molluscum contagiosum infection occurs worldwide and is spread through mild skin trauma, fomites, and sexual transmission.
- Yatapoxvirus infections are rarely reported; infections are acquired through exposure to infected animals and potentially arthropod vectors and are usually geographically restricted to Central Africa and East Africa.

Microbiology
- Poxviruses are a diverse group of large, complex double-stranded DNA viruses that replicate in the cytoplasm of the host cell.

Diagnosis
- Parapoxvirus, molluscum contagiosum, and yatapoxvirus infections are often diagnosed clinically based on their characteristic clinical features in combination with appropriate exposure and travel histories.
- Diagnostic laboratory testing for poxviruses may include polymerase chain reaction assay, electron microscopy, viral culture, and serology.

Therapy
- Parapoxvirus infections are usually self-limited; treatment with topical and intralesional cidofovir as well as imiquimod has been anecdotally reported.
- Multiple modalities for treatment of molluscum contagiosum have been described including cryotherapy, mechanical curettage, and chemical treatments with podophyllin-podofilox, cantharidin, iodine, and tretinoin.

Prevention
- Transmission of parapoxviruses, molluscum contagiosum, and yatapoxviruses can be prevented by avoiding exposures to animal vectors, properly covering lesions, and observing good hand hygiene practices.

PARAPOXVIRUSES

Parapoxviruses, which are found worldwide, are common pathogens of sheep, goats, and cattle. Human infection, characterized by localized epithelial lesions, is an occupational hazard for people who handle infected animals. Parapoxvirus infection in sheep and goats is usually referred to as *sore mouth, scabby mouth, contagious pustular dermatitis/ecthyma,* or *orf,* and the corresponding human infection is referred to as *orf.* Taxonomically the relevant parapoxvirus species is referred to as *Orf virus*; synonyms are contagious pustular dermatitis virus and contagious ecthyma virus. Parapoxvirus infection of dairy cattle is usually referred to as *paravaccinia, pseudocowpox,* or *ring sores,* and the human equivalent is referred to as *paravaccinia, pseudocowpox,* or *milker's nodes.* The virus species is referred to as *pseudocowpox virus*. Parapoxvirus infection of beef cattle is referred to as *stomatitis papulosa.* The parapoxvirus species associated with beef cattle is referred to as *bovine papular stomatitis virus*. Other zoonotic infections caused by tentative members of the *Parapoxvirus* genus and shown to infect humans are derived from camel exposure (contagious ecthyma or Ausdyk disease) or, less often, from seals (sealpox) and deer.[1,2,3] The parapoxvirus of red deer in New Zealand is another parapoxvirus species. Lesions in animals are found on the skin, in the oropharyngeal mucosa, and on external surfaces. Other parapoxviruses documented to cause ungulate, pinniped, or other nonhuman animal infections may also cause human illness. Detailed reviews of members of this genus of Poxviridae are available.[4–7]

Morphology and Composition of the Agent

The parapoxviruses have a unique appearance among poxviruses, as revealed with negative-stain transmission electron microscopy. Most poxvirus virions are brick shaped, but parapoxvirus particles are oblong, rounded, or ovoid. In addition, members of the *Parapoxvirus* genus have a characteristic M form that can be observed with negative-stain electron microscopy: one long spicule wraps the particle, giving a crisscross effect.[8] The stability of the virus in scabs is correlated with possible transmission of the virus through fomites.

The parapoxvirus genome consists of a linear double-stranded DNA of about 135 kilobase pairs with covalently closed terminal hairpins; the genome is relatively high in G+C content and is smaller than other poxvirus genomes. Complete genome sequences of Orf virus and bovine papular stomatitis virus have been reported.[9] Several proteins have been described to have potential roles in viral pathogenesis[10] including a chemokine-binding protein,[11] an interleukin-10 homologue,[12] a vascular endothelial growth factor homologue,[13] an interferon resistance gene,[14] and a cytokine-binding protein.[15]

Pathogenesis and Immune Response

Infection, which occurs via cuts and scratches, usually remains localized in the epithelium or oral mucosa. Human lesions of orf are produced by hypertrophy and proliferation of epidermal cells, which is often marked and perhaps related to the endothelial growth factor homologue encoded by the virus and to leukocyte infiltration. Histologic examination of human lesions shows many small multilocular vesicles within the dermis; true macrovesicles rarely occur.[16,17] Generalized symptoms of lymphadenopathy, malaise, and disseminated lesions are uncommon, and the immune response is not protective against disease recurrence on a lifelong basis.[17,18] Second attacks occur in 8% to 12% of individuals.[17,19]

Clinical Manifestations

Detailed descriptions of human disease progression are available,[16–18] as are illustrations (Fig. 133.1).[20,21] In brief, infection manifests as localized lesions at the site of inoculation by a diseased animal. The portal of entry is usually a break in the skin. Six stages of clinical disease are

FIG. 133.1 Human orf virus infection. Nodule caused by orf virus after contact with a lamb being sacrificed for a holiday—Massachusetts 2010. *(From Centers for Disease Control and Prevention. Human orf virus infection from household exposures—United States, 2009-2011.* MMWR Morb Mortal Wkly Rep. *2012;61:245–248.)*

described. After a brief incubation period of 3 to 5 days, lesions begin as (pruritic) erythematous macules and then raise to form papules, often with a target appearance (days 7–14). Lesions become nodular or vesicular, and orf lesions often ulcerate after 14 to 21 days; this ulceration has been referred to as the *acute stage.* Complete healing can take 4 to 6 weeks and is characterized by a regenerative papilloma and regressive stages where normal epithelium is seen once again.[17] Very large granulomatous lesions occur, more frequently in individuals who are immunocompromised, and these may need surgical removal; other, less invasive therapeutic methods may now be feasible and are discussed later in this chapter.[22] Milker's node lesions may have a more nodular appearance, without ulceration. Parapoxvirus infections reported in handlers of reindeer and musk-oxen in Norway are more granulomatous and persist for months.[23] Erythema multiforme and Stevens-Johnson syndrome have been associated with parapoxvirus infections.

Diagnosis

Polymerase chain reaction diagnostic tests generic for parapoxvirus[24,25] and orf[25,26] have been reported; however, the infection is usually clinically diagnosed on the basis of exposure history and the presence of a characteristic lesion. Negative-stain transmission microscopy of lesion material examined by a skilled observer can be diagnostic if the characteristic structure is observed. Virus isolation in tissue culture usually requires primary ovine or bovine cells and may be difficult to attain.[27] The development of immunologic sera specific for parapoxviruses is another source for diagnostic reagents.[28]

Epidemiology

Infection with parapoxvirus is an occupational hazard of farm workers, abattoir workers, veterinarians, students, and others with frequent exposure to sheep, cattle, or goats. Human orf infection is most common in the spring, a time when the bottle-feeding of lambs may predispose humans to exposure risks, and in the fall, when slaughtering and shearing occur.[17] Of 191 cases of orf or milker's nodule with a known source surveyed from 1978–95, 84% had an ovine source, and 16% were transmitted by cattle. An additional 32 cases occurred in abattoir workers.[29] Human orf infections have also been associated with exposures during household meat processing or animal slaughter, particularly in association with religious holidays.[30,31] Another study evaluating a cluster of cases in children in the Midwest United States found that facial contact with infected animals often led to disease acquisition in children.[25] Most workers at risk are infected at some point in their career, and reinfection is not uncommon. Infected individuals should take care not to further infect themselves with autoinoculation or to spread infection to contacts, including animals. The vaccine used to control orf in sheep is fully virulent and has caused human infection.

Therapy

In most cases, the disease is self-limited. Anecdotal reports of the use of 3% cidofovir topical cream[32,33] have described apparent beneficial effects; however, no controlled trials are available. Topical treatment with the Toll-like receptor/interferon modulating compound imiquimod has also shown benefit in a number of cases,[34,35] including the case of a patient with a giant orf lesion that failed to respond to topical and intralesional cidofovir.[36]

MOLLUSCUM CONTAGIOSUM

Molluscum contagiosum, a disease that causes a benign self-limited skin "tumor" or papular eruption, occurs worldwide and is regarded as a specific human infection.[37] Although no evidence exists of disease transmission between humans and other animals, lesions that resemble molluscum and contain pox virions have been detected in species other than humans (e.g., horses and chimpanzees).

Description of the Agent

Four subtypes, characterized by restriction endonuclease digests, have been described.[38] Disease presentation by all subtypes appears to be similar. The genome of molluscum contagiosum virus subtype I has been sequenced.[39] This genome encodes several novel gene products involved in its pathogenesis and in evasion of the immune system including an interleukin-18–binding protein[40] and apoptosis inhibitors,[41] among others.

Pathogenesis and Pathology

Molluscum contagiosum lesions have long been known to have a distinctive pathology. In 1841 the first description of characteristic molluscum bodies—Henderson-Paterson bodies—was provided by Henderson and Paterson. Onset of infection occurs when the virus begins replication in the lower layers of the epidermis[42] and then extends upward. The incubation period is quite variable and can be lengthy (2–7 weeks; as long as 6 months has been suggested). The epidermis hypertrophies and extends down into the underlying dermal strata. Characteristic inclusions (Henderson-Paterson bodies, or molluscum bodies) are formed in the prickle cell layer and gradually enlarge as cells age and migrate to the surface. These cells are replaced by hyperplasia of the basal cell layer. The structure of the basement membrane remains intact; the hypertrophied epidermal cells, with their cytoplasm occupied by a large acidophilic granular mass (the molluscum body), project above the skin to appear as a tumor.[43] Little to no inflammatory infiltrate is seen until late in disease, just before natural resolution of the lesion occurs.[44]

Clinical Manifestations

Infection occurs after breakage of the skin. The characteristic lesion begins as a small papule and, when mature, is a discrete, 2- to 5-mm-diameter, smooth, dome-shaped, pearly or flesh-colored nodule that is often umbilicated (Fig. 133.2). A cheesy off-white, sometimes yellowish, material is easily expressed from lesions. Usually, 1 to 20 lesions occur, but occasionally, hundreds may be seen. Because of multiple simultaneous infections, or mechanical spread, these lesions may become confluent along the line of a scratch, and satellite lesions are occasionally seen.

In children, lesions occur mainly on the trunk and proximal extremities. In adults, they tend to occur on the trunk, pubic area, and thighs, but in all cases, infection may be transmitted to other parts with autoinoculation.[44] In patients infected with human immunodeficiency virus (HIV), molluscum infections occur along the beard line in male patients; with facial involvement, ocular involvement such as lesions on the bulbar conjunctiva has been found.[45] Individual lesions last for about 2 months, but the disease usually persists for 6 to 9 months.[46] Severe and prolonged infection tends to occur in individuals with impaired cell-mediated immunity, including patients with HIV infection.[37,47]

Diagnosis

The clinical appearance of lesions is generally sufficiently characteristic to permit clinical diagnosis. Brick-shaped virions can usually be seen in large numbers if the cheesy material expressed from the lesion is examined with transmission negative-stain electron microscopy. The virus has

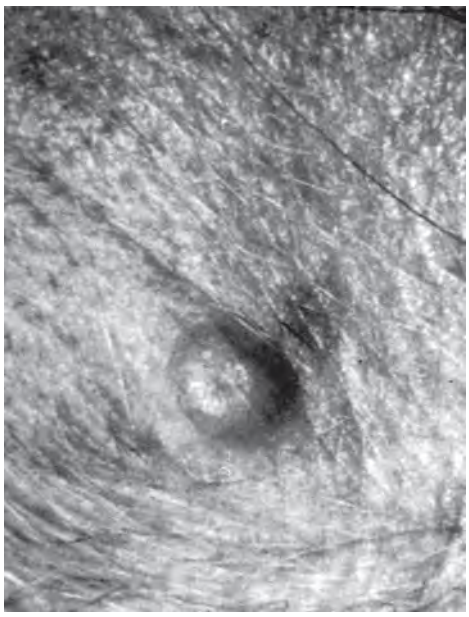

FIG. 133.2 Molluscum contagiosum. *(From Wood MJ. Skin and soft tissue infection. In: Farrar WE, Wood MJ, Innes JA, et al, eds.* Infectious Diseases Text and Color Atlas. *Hong Kong: Gower Medical Publishing; 1992.)*

not been cultured in standard tissue culture systems. The characteristic histopathology of these lesions is diagnostic; polymerase chain reaction methods have been described.[48,49] Similar-appearing umbilicated lesions have occasionally been seen in patients with acquired immunodeficiency syndrome (AIDS) with disseminated cryptococcosis.

Epidemiology and Control

The virus occurs worldwide, and reports of increasing disease have paralleled reports of AIDS. Traditional modes of transmission are associated with mild skin trauma and in some cases fomites (shared towels). Among adults the disease is often sexually transmitted, and genital lesions are common.[37,50] Atopic dermatitis and pubic hair removal, which may result in minor skin trauma or deficits in the epidermis, have emerged as possible risk factors for infection.[51,52,53]

Molluscum contagiosum presents a significant concern for individuals whose children are in daycare or school situations, where concerns about potential transmission to other children may exist. Covering of lesions and hand hygiene after contact with lesions should prevent transmission in these situations.

Therapy

Infection is benign and recovery is usually spontaneous, but treatment may be sought for cosmetic reasons, particularly for facial or multiple lesions. Various treatments have been tried[37] including cryotherapy,[54] mechanical curettage,[54,55] and chemical treatments (podophyllin/podofilox, cantharidin, iodine, and tretinoin).[55–57] Irritation is an adverse effect of many of the chemical treatments. Topical application of an antiviral 3% cidofovir cream or suspension[58,59] has been reported to be beneficial, as has potentially immune-modulating cimetidine.[60] Topical imiquimod therapy has been reported to be beneficial,[61] but two unpublished, large, randomized clinical trials failed to show efficacy.[62] The absence of well-controlled trials makes assessment of the efficacy of various therapeutic regimens difficult for the clinician. A retrospective medical chart review and telephone survey failed to show a beneficial effect of any therapy compared with no therapy on time to clearance or rate of recurrence of lesions.[63] For patients with AIDS and molluscum, the use of highly active antiretroviral therapy, with improved $CD4^+$ cell counts, appears to be efficacious.

YATAPOXVIRUSES

The genus *Yatapoxvirus* includes tanapox virus and Yaba monkey tumor virus.

Tanapox

Human infection with tanapox virus, which was first recognized in the Tana River basin area of Kenya in 1957, was best characterized during post–smallpox eradication surveillance efforts. An account of 264 laboratory-confirmed cases from Zaire (now the Democratic Republic of Congo), with color illustrations, is available,[64] as is information on the virus itself.[65] The genome of the virus has been sequenced.[66] Yaba-like disease virus of monkeys is a variant of the species of virus that causes tanapox in humans.[7,67] Recent anecdotal reports of human disease outside Africa have been published and illustrate the need to consider poxvirus causes of illness in travelers returning from and emigrants from areas where the virus is endemic.[68–70]

Pathophysiology and Clinical Manifestations

Tanapox infection begins with a short febrile illness of 2 to 4 days with temperatures of 38°C to 39°C (100.4°F to 102.2°F) that is sometimes accompanied by headache, backache, or prostration. The eruption of a lesion is often heralded by pruritus at the site. The lesion appears as a hyperpigmented macule, often with central elevation. The macule then evolves to a papule, with palpable induration. Fever and systemic symptoms wane as the lesion manifests. The papule then becomes more "pocklike" but contains no fluid; umbilication or the formation of a pseudocrust has been reported at this stage. Typically the papule evolves into a firm, deep-seated, elevated nodule. At the end of the first week, the lesion is surrounded by erythema and by indurated skin. Regional adenopathy is common at this stage. After this stage, lesions either ulcerate or become larger nodules, up to 2 cm in diameter. In the African series, maximum size was usually reached within 2 weeks, and then the local inflammatory response began to wane and the lesion began to granulate. Resolution of lesions occurred within 6 weeks.[64]

Most cases (78% in one series[57]) involve a solitary nodule; however, as many as 10 lesions on one individual have been described. The most common location for lesions (72%) is the lower extremities, and the least common locations are the face and parts of the body that are normally covered by clothing.[64] Infection appears to confer lifelong immunity.

Diagnosis

For diagnosis of tanapox, the limited geographic distribution should be considered, as should travel history. Unique clinical features that allow the differentiation of tanapox from other orthopoxvirus infections include the nodular nature of the rash lesion, local adenopathy, paucity of lesions, benign disease course, and protracted course of rash resolution. As well, the solid nodular/ulcerated lesions are larger and develop more slowly than the lesions of monkeypox, but they are smaller and develop more rapidly than tropical ulcers.

Tanapox virus can be detected with electron microscopy, and the virions usually appear enveloped.[71] This finding does not exclude the possibility of infection with other morphologically similar brick-shaped poxviruses; nucleic acid testing[68,69] on-lesion extract could be used for that purpose. Tanapox virus grows in a number of cell lines (e.g., owl monkey kidney, Vero, MRC-5, BSC-1), but not on cell adhesion molecules.

Epidemiology and Control

Tanapox virus is restricted to Africa, principally to Kenya and the Democratic Republic of the Congo, and likely has a simian reservoir.[67] Cases of direct primate-to-human transmission, via a break in skin, have been described in animal handlers, although such cases appear to be extremely rare.[72,73] Several factors have led to speculation that an insect or arthropod intermediary may be involved in transmission of tanapox virus to humans. Persons confirmed to have tanapox infection have denied contact with nonhuman primates but have reported arthropod and culicine mosquito bites before infection. Also, in patients in whom multiple lesions developed, no evidence was found that the virus had been spread mechanically.[64] Furthermore, the seasonal variation of human tanapox infections follows the activity of local arthropod populations. No human-to-human transmission has been reported. With the exception of vaccination, measures for the prevention of monkeypox are applicable to tanapox.

Yaba Monkey Tumor Virus

Yaba monkey tumor virus is a distinct species of *Yatapoxvirus*. It was originally isolated as the cause of a cutaneous tumor on a rhesus monkey *(Macaca mulatta)*. In Asiatic monkeys, the virus causes benign histiocytomas that resolve in 1 to 2 months.[74] Serosurveys have suggested that African green monkeys are the natural host of Yaba monkey tumor virus.[75]

Clinical Manifestations

Accidental needlestick infections of human animal handlers and deliberate infections of human volunteers show that humans have localized skin lesions that develop at the site of inoculation.[76] Human infections have not been recently reported.

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The complete reference list is available online at Expert Consult.

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134 Introduction to Herpesviridae

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Although there are hundreds of herpesviruses that infect nearly all animals, eight herpesviruses naturally infect humans (Table 134.1). A ninth herpesvirus, herpes B virus, which naturally infects macaques, can cause fatal encephalitis in humans. This chapter is an overview of herpesviruses that infect humans. Individual herpesviruses are discussed in subsequent chapters (see Chapters 135–141).

CLASSIFICATION

Members of the Herpesviridae are distinguished from other virus families by their structure and genome. Herpesviruses contain double-stranded DNA surrounded by an icosahedral nucleocapsid, which is wrapped inside a tegument consisting of several viral and cellular proteins and then surrounded by an envelope studded with viral glycoproteins (Fig. 134.1).[1] The envelope is derived from host cell membranes. Herpesviruses range from 120 to 260 nm in diameter. Virions contain a characteristic set of viral proteins as well as some host cell proteins.

Human herpesviruses are subdivided into three subfamilies (see Table 134.1). The Alphaherpesvirinae include herpes simplex virus (HSV) types 1 (HSV-1) and 2 (HSV-2), varicella-zoster virus (VZV), and herpes B virus. These viruses are latent in neurons of sensory ganglia, and infection of cultured cells leads to rapid destruction of the cells. They usually cause mucocutaneous infections in healthy individuals. The Betaherpesvirinae include cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), and human herpesvirus 7 (HHV-7). These viruses have a more limited host range, replicate slowly in cell culture, and establish latency in mononuclear cells. The Gammaherpesvirinae include Epstein-Barr virus (EBV) and Kaposi sarcoma–associated herpesvirus (KSHV, also known as HHV-8). These viruses establish latency in lymphoid cells and cause lytic infection in epithelial cells or fibroblasts. The Betaherpesvirinae and Gammaherpesvirinae can cause lymphoproliferation with mononucleosis.

EBV and HHV-6 are present as one of two types, referred to as types A and B. EBV types can differ depending on the geographic location of the infected individual. Although HSV-1 and HSV-2 share 50% or more sequence identity, different types of EBV and HHV-6 are much more closely related.

GENOME STRUCTURE AND PROTEINS

The human herpesviruses (HHVs) consist of 125,000 to 229,000 bp of double-stranded DNA (see Table 134.1), and their overall guanosine and cytosine content varies from 36% (for HHV-7) to 70% (for HSV-2). The genomes consist of long unique regions and short repeated regions. These repeats, as well as single nucleotide polymorphisms, are often useful for molecular epidemiologic studies because restriction endonuclease digestion results in different-sized fragments that can help to indicate whether different persons are infected with different strains of virus. Thus, after transplantation, the sizes of the repeated regions can help to indicate whether the viruses originate from the donor or the recipient.[2] EBV has terminal repeats at the ends of its genome. The number of terminal repeats is fixed in cells that were infected with the same clone of virus but varies in number if the cells were infected with different viral clones.[3] The viral genome is linear inside the virion but circularizes in infected cells. Portions of many herpesvirus genes overlap in the genome; most herpesvirus genes are not spliced.

HHVs encode 71 to several hundred genes.[4] Herpesviruses encode a core set of approximately 40 proteins that are conserved among all the herpesvirus species and include proteins involved in nucleic acid synthesis (e.g., viral DNA polymerase), nucleic acid metabolism (e.g., ribonucleotide reductase), protein modification (e.g., protein kinases), and virion structure (major capsid protein; glycoproteins B, H, and L). In addition, members of the three subfamilies of the Herpesviridae each contain a conserved set of genes unique to each subfamily, such as genes encoding proteins important for virus entry. Herpesviruses have a lytic phase of replication that results in cell death and a latent phase of replication in which no or a very limited set of viral proteins are made.

VIRUS REPLICATION

Herpesviruses use at least two principal methods to enter cells.[5] Virus can enter cells by endocytosis and subsequent fusion of the virion envelope to the endocytic membrane, which allows entry of the nucleocapsid into the cytoplasm. Alternatively, the virion envelope can fuse to the cell membrane on the cell surface to deliver the nucleocapsid directly to the cytoplasm. Viral glycoproteins bind to receptors on the cell membrane, which allows entry into the cell. Herpesviruses often have more than one cell surface receptor (see Table 134.1). The viral nucleocapsid is transported from the cytoplasm to the nucleus, where the linear viral DNA circularizes, and thus DNA replication can begin. Replication proceeds in an orderly pattern of viral gene expression. The immediate-early viral genes are initially expressed; they encode proteins that regulate viral gene expression. This is followed by the early viral genes, many of which encode enzymes important for viral DNA replication or protein phosphorylation. Last, the late viral genes are made, many of which encode structural proteins, including the viral glycoproteins and nucleocapsid proteins. Viral genes are transcribed in the nucleus, and the proteins are synthesized in the cytoplasm. Herpesvirus nucleocapsids are assembled in the nucleus and undergo envelopment at the inner nuclear membrane, deenvelopment at the outer nuclear membrane, and reenvelopment at the cytoplasmic membrane, and then exit the cell. Lytic replication of herpesviruses inhibits host cell RNA and protein synthesis.

VIRUS LATENCY AND REACTIVATION

The mechanisms by which herpesviruses establish and maintain latency are not well understood. Some viruses, such as HSV, express one family of latency-associated transcripts that do not encode proteins but may be important to prevent apoptosis.[6] CMV expresses multiple viral RNAs.[7] Other viruses express proteins during latency. EBV expresses the EBV