covering the major HTLV-1–endemic areas have demonstrated that variations are more linked to the geographic origin of the infected individuals than the patient's clinical status.[124,125] In addition, simian T-lymphotropic virus type 1 (STLV-1) isolates cluster phylogenically more closely with human isolates in the same geographic region than with STLV-1 isolates from different geographic regions, suggesting multiple instances of transspecies transmission.[126–129] Thus the genetic variability of HTLV-1 appears to reflect a combination of multiple transspecies transmission episodes and migration patterns of ancient populations carrying the virus.

An African origin of most HTLV-1 subtypes is supported by the occurrence of many HTLV-1 clusters in Africa[61,73,130] and among persons of African descent residing in the Caribbean, but not among other migrant populations residing in the region. The cosmopolitan subtype was likely dispersed throughout the world during the slave trade or early maritime explorations of European countries. The high prevalence of this HTLV subtype in southwestern Japan remains unexplained and could be due either to importation by maritime trade with Portugal and Holland starting in the 16th century or to prehistoric dissemination of the cosmopolitan subtype among some ancient ethnic people of Japan.[131–133]

HTLV-1 subtype c was first isolated from seropositive individuals in various parts of Melanesia.[134] The original isolate was obtained from an unacculturated hunter-gatherer tribe, the Hagahai, residing in the highlands of Papua New Guinea. More recent work has identified a high prevalence of HTLV-1c among Australian aborigines, with differences in viral sequence between northern and southern Australia.[119] HTLV-1 subtype c clusters closely with STLV-1 isolated from a stump-tailed macaque *(Macaca arctoides),* providing strong evidence of multiple cross-species transmissions.[135] In some cases, these strains have been isolated from persons with ATL[124] and HAM.

HTLV-2

Compared with HTLV-1, the geographic origin of HTLV-2 is less certain. HTLV-2 was first documented in and is highly prevalent among intravenous drug users in the United States and Italy.[109,136–138] Endemic foci of HTLV-2 were subsequently discovered among various Amerindian populations residing in North, Central, and South America.[114,139,140] The current distribution of HTLV-2 in the New World probably represents an original transmission from an endemic Amerindian to an intravenous drug user, followed by amplification of prevalence by the efficient transmission within the latter group. The presence of HTLV-2 infection in culturally and geographically distinct Indian groups in North America, Central America (Panama), and South America (Argentina, Brazil, Colombia, and Chile) led to the speculation that HTLV-2 may have originated in the New World. However, New World monkeys do not harbor STLV-2, and the discovery of HTLV-2 among Central African pygmies has discounted this hypothesis.[106,112]

Based on the relative divergence of nucleotide sequences of the *env* and *LTR* regions, HTLV-2 is classified into four subtypes: HTLV-2a (previously known as HTLV-2 Mo), HTLV-2b (formerly HTLV-2 NRA), HTLV-2c, and HTLV-2d.[141,142] Molecular epidemiologic studies have shown that HTLV-2a is the predominant infection among intravenous drug users in urban North America and among some North American Amerindians.[143] HTLV-2b is the predominant subtype in Indian groups in Panama, Colombia, and Argentina but is also found among Bakola pygmies in Cameroon and European intravenous drug users.[106] HTLV-2c appears to be confined to Brazilian Indian and urban populations and is supported as a separate subtype by *LTR* region phylogeny but not *env* gene phylogenic analyses. HTLV-2d was found in an Efe pygmy in the Democratic Republic of Congo and is the closest relative to STLV-2 isolated from Bonobo chimpanzees *(Pan paniscus).*[112]

Virus isolates from these populations have revealed that HTLV-2a and HTLV-2b differ molecularly by 2% to 4%.[144] The TAX-2 protein of HTLV-2b is 25 amino acids longer and is a more potent transactivator of the HTLV-2 LTR than the corresponding HTLV-2a protein.[142] The in vivo significance of this functional difference is unknown. Conversely, HTLV-2c has LTR and *env* sequences related to HTLV-2a and *tax* sequences similar to those of HTLV-2b. The identical sequence of the 456-bp *tax* gene of HTLV-2b isolates from a Cameroonian pygmy and a Colombian Wayuu Indian support an African origin for HTLV-2.[141] This, taken together with the close similarity of HTLV-2d and STLV-2, supports a scenario wherein HTLV-2 evolved into subtypes a, b, and d in Africa and was then transported to the New World, whereas HTLV-2c represents a more recent variant within subtype a.

HTLV-3 and HTLV-4

Two additional HTLV types have been isolated from Central Africans living in close contact with nonhuman primates. These viruses were discovered simultaneously in 2005 by two teams working on samples derived from two inhabitants of the rain forest area in South Cameroon. Wolfe and colleagues[6] described two different viral isolates from bushmeat hunters, which they named HTLV-3 and HTLV-4. Calattini and colleagues[7] described an HTLV-3 isolate that seems to be identical to the HTLV-3 described by Wolfe and colleagues.[6] Only three additional instances of HTLV-3 human infections and two additional instances of HTLV-4 human infections have been reported, all from Cameroon.[11,145,146] Simian STLV-4 isolated from two gorillas show nucleotide sequences virtually identical to human HTLV-4 isolates.[147] Therefore, reported isolates of HTLV-3 and HTLV-4 probably represent limited instances of simian-to-human transmission that have not become established in the human population.[148] There has been no evidence that either new HTLV type is widely prevalent in Africa or present at all outside Africa, and current commercially available tests have not yet been adapted to detect these new HTLV types.

ROUTES OF TRANSMISSION

Table 168.2 summarizes the routes, mechanisms, and cofactors associated with HTLV-1 and HTLV-2 transmission. The three major routes of HTLV-1 transmission are mother-to-child transmission, sexual transmission, and parenteral transmission. Although the routes of HTLV-2 transmission have been less well studied, the available evidence suggests that they are similar to those of HTLV-1. Transmission involving casual contact does not seem to occur. Zoonotic transmission of HTLV-1 continues to be reported in association with nonhuman primate bites in Central Africa.[149,150] These include, most frequently, bites from gorillas but also chimpanzees and small monkeys.

TABLE 168.2 Transmission of HTLV-1 and HTLV-2

	HTLV TYPE 1	HTLV TYPE 2
Route of Transmission		
Mother to child		
Transplacental	Low efficiency	Probable, but not quantified
Breast milk	Efficient	Probable, but not quantified
Sexual		
Heterosexual	Efficient	Efficient
Male to male	Efficient	Unknown
Parenteral		
Blood transfusion	Very efficient	Very efficient
Injection drug use	Efficient	Efficient
Cofactors of Transmission		
Elevated virus load		
Mother to child	Increased	Unknown
Heterosexual	Increased	Increased
Sexually transmitted diseases	Increased	Unknown
Cellular versus plasma transfusion products	Increased	Increased
Cold storage of blood	Decreased	Decreased

HTLV, Human T-cell leukemia virus.

Mother-to-Child Transmission

In contrast to mother-to-child transmission of HIV-1, wherein as many as 25% of offspring of positive mothers can acquire infection without breastfeeding,[151] breastfeeding is the predominant route of mother-to-child HTLV-1 and HTLV-2 transmission[152,153] and occurs through ingestion of infected milk-borne lymphocytes.[154] Both HTLV-1 and HTLV-2 viruses have been detected in breast milk.[155,156] During the first 6 months of life, maternal antibodies are present in infants; in serial WBs, all bands often disappear before new bands appear as a result of neonatal infection. In some cases, breastfeeding had ceased up to several months before seroconversion, but a study of cells from exposed but nonseroconverting children identified none with latent HTLV-1 viral infection.[66] In Japanese intervention trials,[152] 20% of breastfed infants seroconverted to HTLV-1, whereas only 1% to 2% of bottle-fed infants of HTLV-1–positive mothers became infected.[152] In prospective studies from Jamaica, a similar rate of transmission has been documented.[155]

The major predictor of maternal-to-child transmission is the proviral load of the mother as measured by means of antibody titer and viral antigen level on short-term culture.[71,157] The presence of antibody to HTLV Tax and/or Env has also been associated with transmission.[155,158] Furthermore, the duration and timing of breastfeeding were strongly associated with the efficiency of transmission.[154,159,160] In a prospective study conducted in Jamaica, among children born of HTLV-1–positive mothers in follow-up for more than 2 years, 32% of children breastfed for 12 months or longer were HTLV-1 seropositive compared with 9% of those breastfed for less than 12 months.[154] These data suggested that limiting the duration of breastfeeding to less than 6 months may reduce mother-to-child transmission of HTLV-1, and such a recommendation has been included in public health advisories. However, a study documented HTLV-1 transmission in 2 of 25 infant-mother pairs with short-term (<3 months) breastfeeding.[161] The same study reported that once initiated, breastfeeding was difficult to stop at 3 months. Follow-up studies indicated that seroconversion typically occurs in infants at the age of 1 to 3 years, with 2% to 5% of HTLV-1 infections resulting from mother-to-child transmission in the first few years of life.[162,163] In many studies, no infants or children became newly infected after 2 years of age.[153] Infection in early life may have considerable significance for subsequent risk for disease, particularly ATL.[164,165]

HTLV-2 transmission from mother to child also appears to occur through breastfeeding but has been less studied.[166] In a small study of infected mothers, 1 of 7 breastfed children was seropositive for HTLV-2, whereas 1 of 28 nonbreastfed children was seropositive.[167] In another study, no HTLV-2 transmissions were found among 19 infected mothers and 20 nonbreastfed children.[168] Among the Gran Chaco Indians of Argentina and the Kayapo Indians of Brazil, high rates of mother-to-child transmission have been observed (30% and 46%, respectively), suggesting the predominant role of breastfeeding in maternal-to-child transmission in these populations.[169] In addition, among the Guaymi Indians of Panama, there is a 1% to 2% prevalence among preadolescent children.[170] This is consistent with early-life infection and an excess of seropositive children when the mother is seropositive compared with the virtual absence of seropositive children when the mother is seronegative.[162,165]

Sexual Transmission

Several markers of sexual activity have been associated with transmission of HTLV-1 infection, including the number of lifetime sexual partners[91,171] and ulcerative (syphilis, herpes simplex virus type 2, and chancroid) and nonulcerative (gonorrhea and *Chlamydia*) sexually transmitted diseases.[91,172–174] Virus-positive mononuclear cells have been detected in semen, which suggests that seminal fluid is a likely vehicle for transmission. The presence of HTLV-1 DNA has also been found in cervicovaginal secretions from infected commercial sex workers in Peru.[175,176] Although some studies have suggested that male-to-female transmission occurs more efficiently than female-to-male transmission,[177,178,179] this difference may be less than previously thought.[180] In a 10-year follow-up study of 30 discordant heterosexual couples, the incidence of HTLV-1 infection was 0.9 per 100 person-years (95% confidence interval [CI], 0.1–3.3), with one male-to-female and one female-to-male transmission.[180] Male-to-male sexual transmission may also occur and is supported by the higher prevalence of HTLV-1 among men who have sex with men (15%) in Trinidad compared with the general population prevalence of 2.4%.[171] HIV-1 shares these routes of infection but appears to be an order of magnitude more infectious than HTLV-1.[171,172] This may reflect differences in viral load or that HTLV-1 is highly cell associated, whereas HIV-1 is both cell associated and cell free.

There are also other risk factors associated with sexual transmission of HTLV-1. In a study of married couples, there was a nearly 12-fold higher risk for infection in wives of seropositive husbands older than 60 years, possibly because of increased viremia with age or postmenopausal changes in the vaginal epithelium.[177] Higher proviral load and duration of relationship are also associated with increased transmission. In a prospective study of HTLV-infected men and their female sex partners, transmitters had higher proviral loads and had been in their relationships longer than nontransmitters.[178] Another prospective study demonstrated that HTLV-1 proviral loads were two $\log_{10}$ lower in newly infected partners than in their positive partners who transmitted HTLV, suggesting that a small dose of sexually transmitted HTLV produces a lower proviral load setpoint.[180] In addition, the presence of anti-TAX antibody has been shown to be associated with heightened transmission, possibly related to a state of virus proliferation induced by TAX and measured indirectly by anti-TAX antibody. Host-related factors may also play an important role in determining HTLV-1 proviral load. A study evaluating the sequence of the gp46 coding region among 13 infected patients and their partners revealed that, although the gp46 sequences were identical in each married couple, the level of HTLV-1 proviral DNA in the spouses often differed.[181]

Sexual transmission of HTLV-2 has been difficult to study because of the frequent coincidence of injection drug use in the study populations. However, a 10-year cohort study of 55 serodiscordant heterosexual couples found two HTLV-2 transmissions, with one male-to-female and one female-to-male transmission (0.5 per 100 person-years), which was not statistically different from rates seen for HTLV-1.[180] In this study, all newly infected sex partners denied any history of intravenous drug use or other parenteral exposure. Previous studies have documented an association between a history of receiving money for sex, total lifetime sex partners,[182] and length of sexual relationships with HTLV-2 seropositivity.[178,179,182] In one study of female prostitutes, intravenous drug use was the major risk factor for seropositivity.[183] In a serosurvey of Guaymi Indians, it was demonstrated that among women, early age at first intercourse (<13 years), higher number of lifetime sexual partners, and higher number of long-term sexual relationships were significantly associated with HTLV-2 positivity.[184] Among men, intercourse with prostitutes was associated with HTLV-2 seropositivity.

Parenteral Transmission

Parenteral transmission, either through transfusion or injection drug use, is another major route of HTLV transmission. Because the HTLV viruses are cell associated, transmission via transfusion of cellular blood components (e.g., whole blood, packed cells, platelet concentrates) is highly efficient. Seroconversion rates of 44% to 63% have been reported in recipients of HTLV-1–infected cellular blood components in endemic areas,[185,186–188,189] but seroconversion has not been associated with plasma or cryoprecipitate. Donor units of whole blood or packed cells are less likely to be associated with transmission the longer they are stored in the blood bank, presumably because of the loss of white blood cell viability at refrigerator temperature. In retrospective surveys, the rate of transmission decreased to near zero when blood components were stored for more than 14 days compared with 47% transmission for a storage period of 14 days or less.[48,186] Removal of leukocytes from blood products via filtration or leukoreduction also appears to substantially decrease but not eliminate the risk of HTLV-1 transmission by transfusion.[190] There is only rare evidence for transmission of HTLV-1 by needlesticks or other bloody occupational exposures.[191,192]

Transmission of HTLV-2 has been documented in 50% of the recipients of known units of positive blood.[193] Parenteral drug abuse has been associated with transmission of both HTLV-1 and HTLV-2, but most HTLV-positive drug abusers are HTLV-2 infected.[194] Risk factors for seroconversion include sharing of drug abuse paraphernalia and "booting"—that is, aspirating blood into the drug-filled syringe

before injection. This circumstance may help to explain the exceptionally high rates of seropositivity in older heroin-injecting drug users, suggesting a birth cohort effect linked to an HTLV-2 epidemic among US injection drug users in the 1960s and 1970s.[194]

Although rare, HAM and ATL have both been linked to HTLV-1 transfusion transmission.[195] In recognition of the risk for these diseases, testing of donated blood for HTLV viruses has been routine in the United States since 1988.[55] HTLV seroprevalence is generally low among hemophiliacs unless they were multiply transfused with cellular blood products before the institution of routine screening. In developing countries, however, blood transfusions remain a major risk factor for HTLV infection. Several cases of HTLV-1 infection in transplant recipients have been documented.[196] These infections have resulted in T-cell lymphomas[197] and in myelopathy.[198] In a cohort in which the diagnosis of HTLV-1 was made soon after transplantation, early treatment with zidovudine and raltegravir was ineffective at controlling viral replication.[199]

IMMUNOLOGY OF HTLV INFECTION

Humoral Immune Responses

Antibodies to the various antigens of HTLV-1 occur at high levels in carriers and among patients with ATL and HAM. During primary infection, the pattern of antibody responses (Fig. 168.7) demonstrates that the first specific antibodies to emerge after primary HTLV infection are directed against the gp21 transmembrane ENV protein and various regions of the GAG polyprotein. Over several weeks to months, anti-gp46 ENV antibodies appear, and about 50% of infected individuals develop detectable antibodies to p38/40 TAX protein.[200] Antibody titers vary from patient to patient and are significantly higher in patients with HAM and among those at risk for this disease. Antibody titers correlate with the proviral burden, which explains the HAM finding and the paradoxical finding of high antibody titers among women who transmit HTLV-1 to their infants through prolonged breastfeeding.[155] Transplacental maternal antibodies apparently protect the infant from infection in the first months of life, but subsequently the infant becomes infected via maternal virus in the breast milk. Miyoshi and coworkers[201] have shown that passive immunization with high-titer human anti-HTLV globulin is protective against experimental HTLV infection in rabbits. Antibody response is also elicited by immunization with experimental DNA or peptide HTLV-1 vaccines.[202] However, there is little evidence that humoral immune response controls HTLV-1 proliferation or prevents disease in humans.

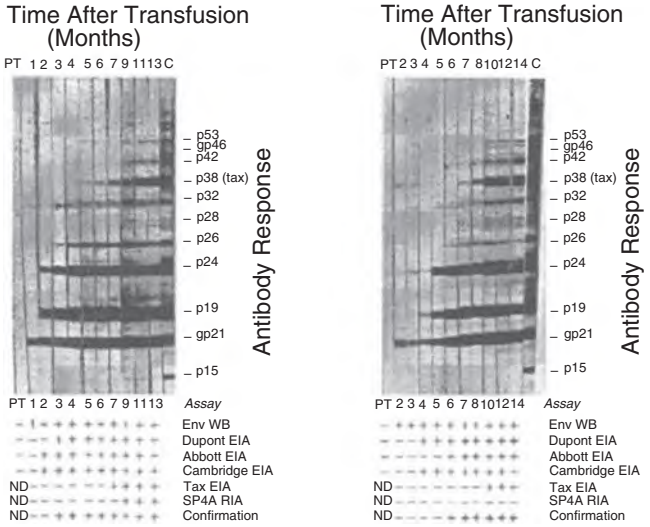

FIG. 168.7 Sequential serum samples from two transfused patients after exposure to human T-cell leukemia virus type 1 (HTLV-1)–infected blood products. The time course (in months) of seroconversion is revealed by the sequential appearance of bands on a standard HTLV-1 Western blot *(top)* and reactivity (+/–, ND) *(bottom)* on a battery of other Western blot *(WB),* enzyme immunoassay *(EIA),* and radioimmunoassay *(RIA)* tests. *ND,* Not done; *PT,* pretransfusion.

Cellular Immunity

Cytotoxic T lymphocytes targeting viral antigens play an essential role in the regulation of HTLV-1 viral burden.[203] Among chronic carriers, infected individuals mount a strong cell-mediated immune response to the virus, and as many as 1% of $CD8^+$ cytotoxic T lymphocytes can recognize at least one epitope of HTLV.[204–209] Freshly isolated cells have substantial expression of activation markers, indicating that these cells have recently encountered the TAX antigen.[203,205,206] A proposed model of this viral interaction with the host cell–mediated immune response is shown in Fig. 168.8. In this model, the dynamic equilibrium between viral replication and immune destruction is mediated through TAX overexpression, causing $CD4^+$ target cell proliferation and a robust cell-mediated response to the antigen in particular, resulting in cytotoxic T-lymphocyte–mediated lysis of these HTLV-1–infected $CD4^+$ cells.[205,206,209,210] As a consequence of ongoing TAX proliferation, there is an expansion of HTLV-1–infected $CD4^+$ cells and a compensatory expansion of $CD8^+$ cytotoxic T lymphocytes. As the number of infected $CD4^+$ cells expands, HTLV-1 antigens are expressed on the cell surface and become targets for $CD8^+$ T-cell–mediated cytotoxic killing. The role of $CD8^+$ T-cell–mediated killing as the primary means of viral suppression may explain the epidemiologic observation that recipients of HTLV-1–infected blood products who are also receiving exogenous immunosuppressive medications are more susceptible to infection, likely because a diminished cell-mediated immune response is unable to clear the initial virus infection.[189]

The role of $CD4^+$-mediated Th1 responses in upregulating the cytotoxic T-lymphocyte response is not well characterized. However, an association between class I human leukocyte antigen (HLA) haplotypes and protection against HAM suggests that carriers of certain antigen-presenting motifs augment the efficient control of HTLV-1–containing cells. HTLV-1 carriers with the HLA-A02 haplotype have lower HTLV-1 proviral loads and are less likely to develop HAM, most likely because they are able to mount a stronger cell-mediated immune response to HTLV-1 infection.[209,211,212,213] Recent work has focused on genetic polymorphisms of HLA alleles and associated immune responses with the hypothesis that genetic polymorphisms determine HTLV-1 clinical outcome; low immune responder HLA alleles exhibit no positive immune responses and are permissive to HTLV-1, and high immune responder alleles are nonpermissive to HTLV-1 and exacerbate host inflammatory responses. Macrophage function does not seem to be affected by HTLV-1 infection, although macrophages from HTLV-1–infected subjects produced more CXCL9 and CCL5 and less IL-10 than cells from uninfected controls.[214]

A review of HTLV pathogenesis suggested that a combination of insufficiency of host HTLV-1–specific T-cell response and elevated proviral load might identify persons with high propensity to progress to ATL.[215] The balance of host immune response with HTLV-driven clonal expansion of infected lymphocytes, possibly with aggravating chronic IFN production, may play a role in the pathogenesis of HAM.[216]

CLINICAL MANIFESTATIONS OF HTLV INFECTIONS

Clinical disease associated with HTLV-1 is rare. ATL develops in 2% to 4% and HAM in 1% to 4% of carriers over their lifetime.[217,218] The risk for the development of disease is related to age and route of infection and the immune competency of the host. Acute seroconversion is associated with no recognized clinical syndrome; the time from infection to seroconversion was about 50 days or perhaps shorter with more contemporary assays, as seen with transfusion cases. The time from seroconversion to disease can vary from years to decades, with rare cases of HAM occurring soon after blood transfusion.[200] As summarized in Table 168.3, there is a wide range of clinical conditions linked to HTLV-1, with some caused by virally induced cell transformation and others mediated by immunologic response to the virus.

HTLV-1–Associated Malignancies

ATL was first recognized in Japan in 1977, before the discovery of HTLV-1, as an aggressive leukemia or lymphoma of mature T lymphocytes with varied clinical manifestations: generalized lymphadenopathy, visceral involvement, hypercalcemia, cutaneous involvement, lytic bone lesions, and peripheral blood involvement with cells manifesting

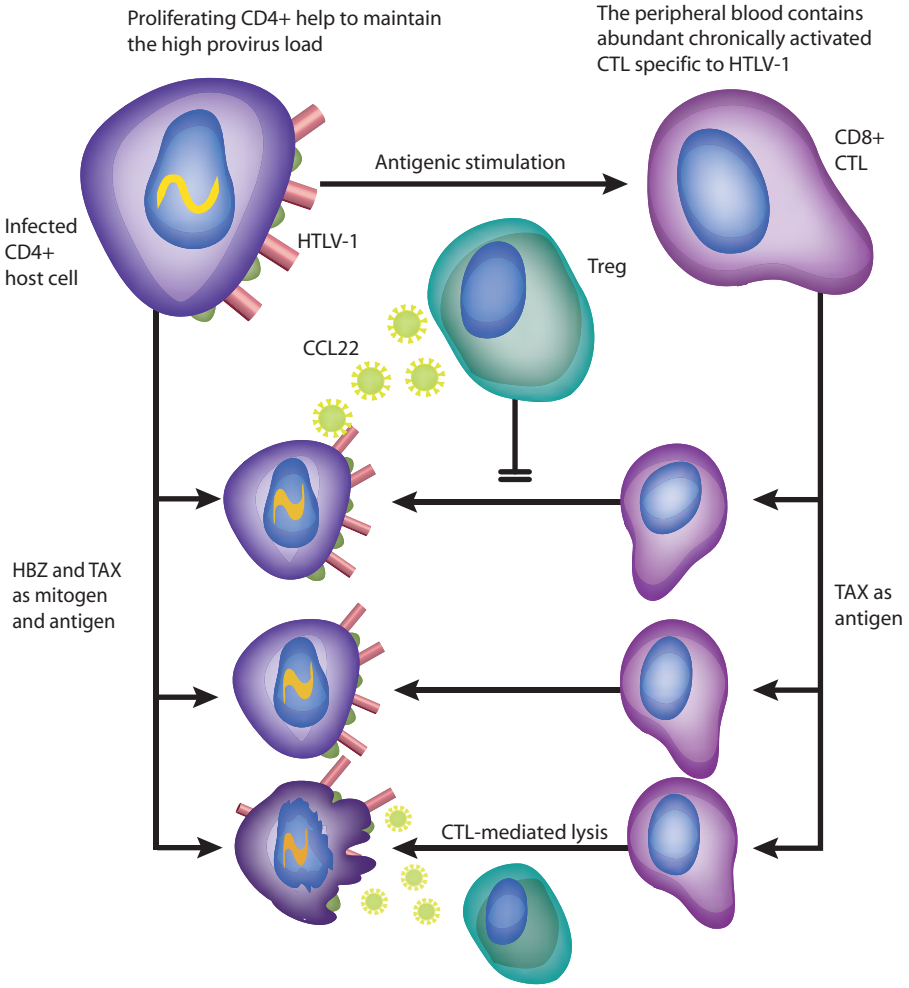

FIG. 168.8 A model of CD8⁺ cytotoxic T-lymphocyte *(CTL)*–mediated control of human T-cell leukemia virus type 1 *(HTLV-1)* infection. HTLV-1 infects CD4⁺ T lymphocytes with expansion of infection primarily via cell replication. As HTLV-1–specific antigens, particularly TAX, are expressed, a robust CD8⁺ cytotoxic T-lymphocyte response is generated. The inability of some persons to control HTLV-1 expansion is believed to contribute to disease pathogenesis. See text for details. *(From Bangham CRM. HTLV-1 infections.* J Clin Pathol. *1999;53:581–586.)*

TABLE 168.3 HTLV-Associated Diseases

	HTLV-1	HTLV-2
Children		
Infective dermatitis	++++	No
Lymphadenopathy	++	++
Adults		
Adult T-cell leukemia/lymphoma	++++	No
HTLV-associated myelopathy	++++	+++
Infective dermatitis	+++	No
Polymyositis	++	Unknown
Uveitis	+++	Unknown
HTLV-associated arthritis	++	++
Sjögren syndrome	++	Unknown
Strongyloidiasis	++	Unknown
Pulmonary infiltrative pneumonitis	++	++
Invasive cervical cancer	+	Unknown

++++, Very strong evidence; +++, strong evidence; ++, possible association; +, weak association; *HTLV,* Human T-cell leukemia virus; *No,* evidence does not support association; *Unknown,* no data to support association or lack of association.

pleiotropic features ("flower cells") in a large number of cases.[219] The skin lesions seen in ATL are varied and include localized or diffuse papules, nodules (Fig. 168.9A), plaques (see Fig. 168.9B), erythematous patches, and diffuse erythroderma. Biopsy specimens of skin lesions reveal dermal or epidermal infiltration with malignant lymphocytes. So-called Pautrier microabscesses may also be noted in the dermis, as in mycosis fungoides. Biopsy specimens of bone lytic lesions reveal osteoclast activation and bone resorption (Fig. 168.10), often without infiltration by ATL cells. It has been suggested that TAX transactivation and production of parathyroid hormone–related protein and other cytokines are responsible for the hypercalcemia, osteoclast activation, and lytic bone lesions seen in this disorder.[220] The lifetime risk for development of ATL in HTLV-1 carriers is estimated at 2% to 4%, and the latent period from infection to development of disease is estimated to be 30 to 50 years.[217,221]

The Lymphoma Study Group in Japan[222] has classified ATL into four clinical types based on clinical features and cell morphology: smoldering (5%), chronic (19%), lymphoma/leukemia (19%), and acute (57%) types. Transformation from the smoldering or chronic phase to the acute form can occur at any point during the course of the disease progression. Fig. 168.11 shows the characteristic morphologic features of the leukemia cells observed in ATL.

Smoldering ATL is characterized by 5% or more abnormal T cells in the peripheral blood with a normal total lymphocyte count, the presence of skin lesions, and, occasionally, pulmonary involvement. There is no hypercalcemia, lymphadenopathy, or other visceral

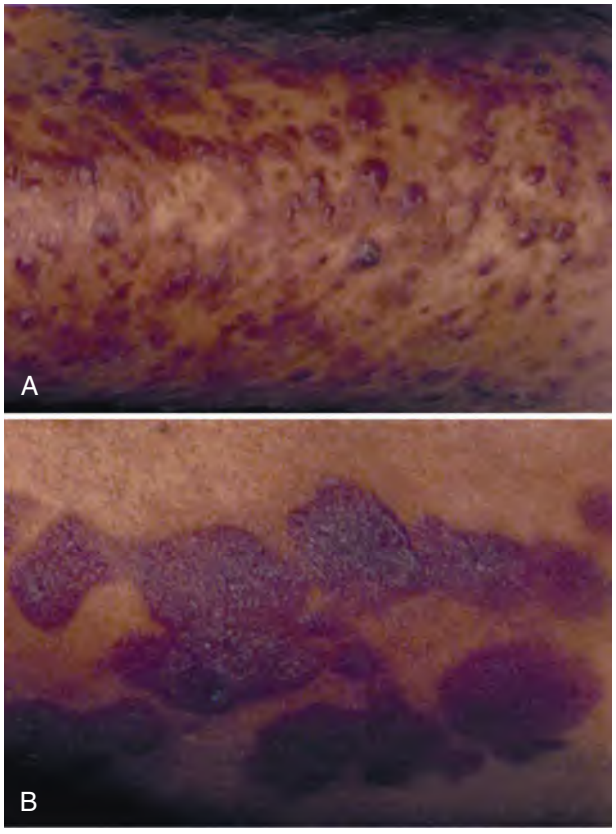

FIG. 168.9 Cutaneous manifestations in patients with adult T-cell leukemia. (A) Multiple skin papules and nodules. (B) Red plaques.

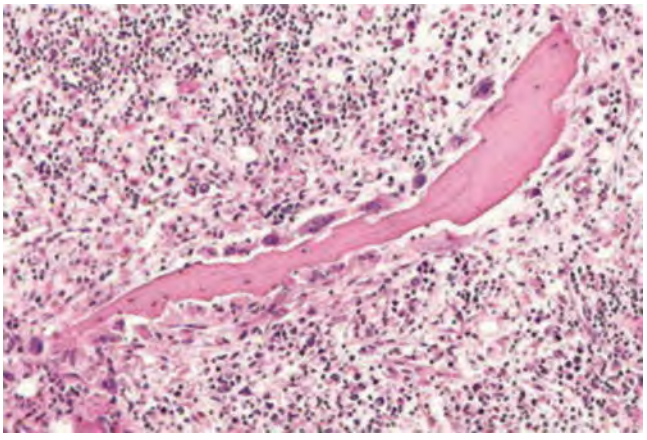

FIG. 168.10 Biopsy specimen of lytic bone lesions from a patient with adult T-cell leukemia reveals osteoclast proliferation and bone resorption.

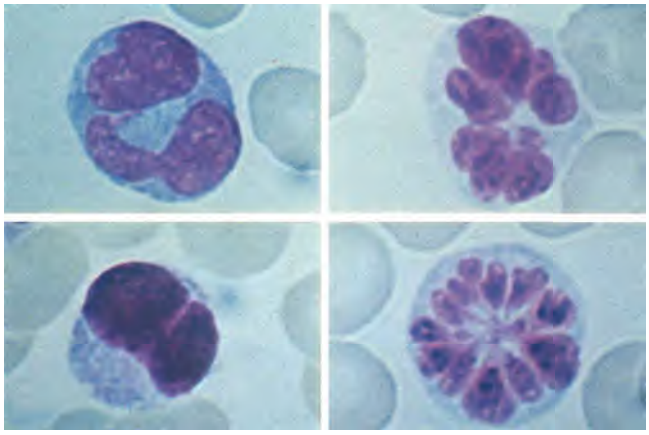

FIG. 168.11 Photomicrographs of typical peripheral blood lymphocytes from patients with adult T-cell leukemia. The characteristic cleaved nucleus can result in either a bilobed shape or the archetypical "flower cell" appearance. Low numbers of flower cells can also be seen in occasional HTLV-1–seropositive patients without any sign of leukemia or lymphoma.

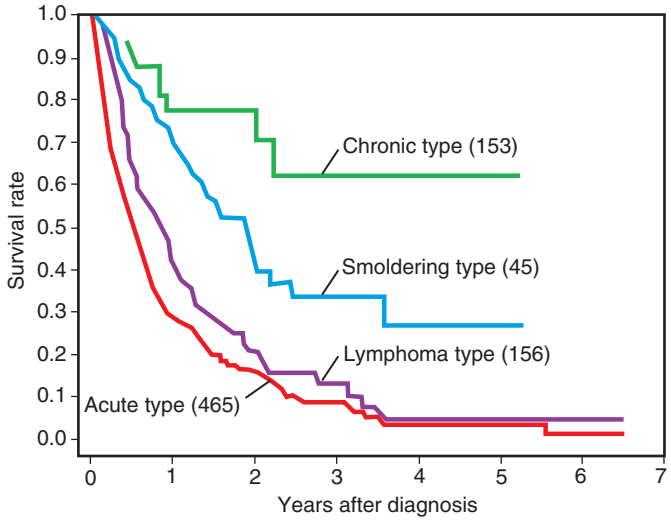

FIG. 168.12 Survival patterns of adult T-cell leukemia (ATL) subtypes after standard chemotherapy. Acute and lymphoma-type ATL have the poorest prognosis (see text). *(From Bazarbachi A, Plumelle Y, Ramos JC, et al. Meta-analysis of the use of zidovudine and interferon-alfa in adult T-cell leukemia/lymphoma showing improved survival in the leukemic subtypes.* J Clin Oncol. *2010;28:4177–4183.)*

involvement. Serum lactate dehydrogenase levels may be elevated. This phase is often indolent and may last for years. Smoldering ATL may clinically resemble mycosis fungoides or Sézary syndrome, with cutaneous involvement manifesting as erythema or as infiltrative plaques or tumors; Pautrier microabscesses may be observed.

Chronic ATL is characterized by an absolute lymphocytosis ($\geq 4 \times 10^9$/L) with a T-cell lymphocytosis ($>3.5 \times 10^9$/L) that resembles chronic T-lymphocytic leukemia. Lactate dehydrogenase level may be increased as much as twice the normal limit. Cells from chronic ATL patients are relatively uniform in size and nuclear configuration. Patients may have lymphadenopathy, hepatomegaly, splenomegaly, and skin and pulmonary involvement. No hypercalcemia, ascites, pleural effusion, or involvement of the central nervous system (CNS), bone, or gastrointestinal tract is present. The median survival time for patients with chronic ATL is 24 months.

Lymphoma/leukemia ATL is characterized by lymphadenopathy in the absence of lymphocytosis. Lymph node involvement with ATL must be histologically proved. The median survival time is approximately 10 months.

Acute ATL is distinguished by increased numbers of leukemic T cells with characteristic pleomorphic morphology, skin lesions, systemic lymphadenopathy, hepatosplenomegaly, and metabolic disorders, especially hypercalcemia. Lytic bone lesions and visceral involvement are common. Acute ATL has a poor prognosis, with a median survival time of 6.2 months.

Most patients with the acute and lymphoma types die within 6 months of diagnosis (Fig. 168.12), particularly if hypercalcemia is a presenting sign.[223] In general, the smoldering type is the least aggressive; the chronic type has a relatively poor prognosis, with death occurring within a few years of diagnosis.[222] The cause of death is usually an explosive growth of tumor cells, hypercalcemia, bacterial sepsis, and other infections observed in patients with immunodeficiency. Sometimes ATL manifests

as a T-cell non-Hodgkin lymphoma with no other clinical features of ATL except for monoclonal integration of HTLV-1 in the proviral DNA of the tumor cells. These cases are termed *lymphoma-type ATL* and are indistinguishable from peripheral T-cell lymphomas.

The malignant T cells of ATL are mature (terminal deoxynucleotide transferase negative) $CD4^+/CD8^-$ and have increased IL-2R α-chain (CD25/TAC antigen) expression.[224] All subtypes have a monoclonal integration of HTLV-1 proviral DNA into the cellular genome, indicating that the malignant T cells are monoclonal and originated from a single HTLV-1–infected T cell.[225] Southern blot analyses show frequent detection of defective provirus but fail to demonstrate consistency of HTLV-1 integration site in tumors from different patients, suggesting that integration site does not determine leukemogenesis.[226] More recently, reverse-transcriptase polymerase chain reaction (RT-PCR) assay has been used to determine the specific genomic location of integration, because HTLV-1 integration sites are more likely to occur in gene-coding than non–gene-coding regions of the genome and near transcriptional start sites in leukemic cells.[227–229] However, no specific proviral integration sites have been linked to the development of ATL.[230] Tanaka and colleagues[46] showed multiple, small HTLV-positive T-cell clonal populations in recently infected persons compared with fewer, larger clonal populations in persons believed to have been infected since birth. The dominant theory is that HTLV-1–driven lymphocyte proliferation results in the accumulation of additional somatic DNA mutations at one or more leukemogenic sites, either flanking the HTLV-1 proviral integration site or elsewhere.[231,232] Chromosomal additions and deletions are common in ATL and may be related to prognosis.[233] A large, multicenter study in Japan has revealed that specific mutations and copy number alterations were associated with both ATL subtype and prognosis.[234] Older age and *PLCG1* and *IRF4* mutations were associated with worse prognosis in aggressive ATL, and *TP53* and *IRF4* mutations, *CDKN2* deletions, and *PD-L1* amplifications were associated with shorter survival in indolent ATL. Consistent with this, a second laboratory's analysis of ATL tumors observed increased *IRF4* mutations, and emergence of *K59R* mutations at relapse suggests that *IRF4* for is a driver mutation and potential therapeutic target.[235]

The transcription factor TP53 guards against DNA damage, and mutations of the *TP53* gene have been reported in more than 50% of human cancers. Mutations of *TP53* are not common in ATL, but TAX inhibits the function of *TP53* through other mechanisms.[236–238] Ubiquitination of TAX attenuates its transcriptional activity.[239,240] As opposed to in vitro models of transformation, TAX is not expressed in approximately 60% of ATL cases.[241] Analyses of HTLV-1 provirus in ATL cases showed three mechanisms to inactivate TAX expression: (1) genetic changes of the *tax* gene,[241,242] (2) DNA methylation of the 5′ LTR,[241,243,244] and (3) deletion of the 5′ LTR.[245,246]

Pneumocystis jirovecii pneumonia, cryptococcal meningitis, disseminated fungal infections, and other opportunistic infections are often present and contribute to a rapid progression to death for patients with acute and lymphoma-type ATL. The fact that *Strongyloides stercoralis* seems to be a common concurrent infection[247] has led to speculation that *Strongyloides* infection may be a cofactor in the development of ATL.[248] The state of immune compromise resulting from HTLV-1 infection is not due to the type of immune ablation observed in HIV-1, even though CD4 cells are infected by HTLV-1; rather, the immunodeficiency is associated with rapidly proliferating malignancy, and the pattern of opportunistic infections is typical for those reported in patients with other types of aggressive non-Hodgkin lymphomas. In nonendemic regions, the diagnosis of ATL is suggested by the presence of HTLV-1 antibodies in serum from patients with the characteristic features of T-cell malignancy and is confirmed by the demonstration of monoclonal integration of HTLV-1 provirus in tumor tissue or leukemic lymphocytes. Measurement of HTLV-1 proviral load is not helpful because levels in ATL are not consistently elevated as they are in HAM and therefore do not discriminate among ATL, HAM, or a subset of healthy carriers. In some cases of ATL in patients from high-risk areas with typical clinical features, antibody is absent but a defective integrated virus with retained *tax* function can be detected with PCR.[249]

There is little evidence that HTLV-1 is associated with solid tumors or hematologic malignancies other than ATL.[250] Arisawa and colleagues[251] reported a 6-year prospective study in which HTLV-1 was not associated with an increased risk for cancer mortality. Previous case-control studies that showed associations between HTLV-1 infection and cancer may have been confounded by blood transfusion or genetic predisposition.[252,253] An inverse relationship between HTLV-1 and *Helicobacter pylori* infections has been noted in Japan, suggesting that there may be an interrelationship with gastric cancer in this setting. However, a recent study found a lower risk for gastric carcinoma in HTLV-1 patients.[254]

The differential diagnosis of ATL includes other T-cell malignancies, such as non-Hodgkin lymphoma, mycosis fungoides, and Sézary syndrome. ATL should be suspected in any patient with a T-cell malignancy who is from an endemic population or has risk factors for HTLV-1. The presence of circulating flower cells, hypercalcemia, and skin lesions is highly suggestive. Leukemic cells are characteristically terminal deoxynucleotide transferase negative $CD4^+$ and $CD25^+$. Laboratory detection and confirmation can be based on testing for anti–HTLV-1 antibody or demonstration of monoclonal integration of HTLV-1 proviral DNA in the malignant cells by means of Southern blot or RT-PCR.

HTLV-Associated Myelopathy

HAM, otherwise known as tropical spastic paraparesis, is a chronic progressive demyelinating disease that affects the spinal cord and white matter of the CNS.[255,256,257] The incidence of HAM in HTLV-1 carriers is estimated to be less than 4%.[218] HAM has been linked to blood transfusion, and some cases are acutely progressive.[195,258] Familial clusters have also been reported and may be associated with younger age of onset and slower disease progression.[259] Although HAM mainly affects adults, particularly women, cases occasionally occur in children younger than 10. The overrepresentation of females may reflect a higher occurrence of HAM after sexually acquired rather than vertical infection.[260]

The typical time of disease onset is in the fourth decade of life. The onset is often subtle, and the florid clinical picture of HAM is not always seen at first presentation. A single symptom or physical sign may be the only evidence of early HAM. Patients often present with a stiff gait,[261] progressing (usually slowly) to increasing spasticity and lower extremity weakness,[262] back pain, urinary incontinence,[263] and impotence in men. Patients may report sensory symptoms such as tingling, "pins and needles," and burning. Vibration sense is frequently impaired.[264] Hyperreflexia of lower limbs, often with clonus and Babinski sign, is frequently found. Hyperreflexia of the upper limbs is less common but may occur in severe cases, whereas upper limb weakness is rare. Exaggerated jaw jerk is seen in some patients, and ataxia may develop.

Nuclear magnetic resonance images may be normal or show atrophy of the spinal cord and nonspecific lesions in the brain.[263,265–267] The syndrome is significantly different from classic multiple sclerosis. HAM follows a slow course without the waxing and waning of symptoms, changes in affect, or multiple nuclear magnetic resonance scan abnormalities characteristic of multiple sclerosis. Varying degrees of brain parenchymal degeneration have also been described, with reactive astrocytosis and perivascular mononuclear cell infiltration. These mononuclear cells are predominantly $CD8^+$ lymphocytes,[268] suggesting that an immune mechanism may play a role in the development of HAM. High frequency of cytolytic T cells with specificity directed against major histocompatibility class I–restricted epitopes derived from the TAX protein has also been observed.[269]

It is possible that HTLV-1 induces an autoimmune-like process through molecular mimicry (an autoimmune model) or through indirect effects on immune function (a cytotoxic model). In the former model, it is postulated that HTLV-1 infection activates autoreactive T cells, which then cause autoimmune destruction within the CNS.[270,271] In the latter model, it is postulated that HTLV-1 infects glial cells, which subsequently induces a cytotoxic immune response against these cells, leading to demyelination.[268]

Patients with HAM characteristically have HTLV-1 antibodies or antigens in the blood and cerebrospinal fluid (CSF). The CSF may show mild lymphocytic pleocytosis; lobulated lymphocytes with morphologic similarity to ATL cells (flower cells) (see Fig. 168.11) also may be present in the blood and CSF.[262] Mild-to-moderate increases in protein may be observed in the CSF, and oligoclonal bonds with specific reactivity to

HTLV-1 antigens have been detected.[261,262] Lymphokines such as IL-6, tumor necrosis factor-α, and IL-2 are increased in the CSF. However, attempts to document the presence of HTLV-1 in the demyelinated lesion have not demonstrated a direct role of the virus in neurons.

The differential diagnosis of HAM includes multiple sclerosis, spinal cord tumors or extrinsic compression, toxic neuropathies, malnutrition, and HIV infection or syphilis. The diagnosis is suspected in unexplained CNS disease with loss of pyramidal tract functions and is confirmed by testing sera for HTLV-1 antibodies. In contrast to lymphocytes in ATL, HTLV-1–infected lymphocytes in HAM are oligoclonal or polyclonal rather than monoclonal.[272]

Neurologic abnormalities other than HAM have also been reported with HTLV-1 infection, including sensory neuropathy, gait abnormality, bladder dysfunction, erectile dysfunction, amyotrophic lateral sclerosis, mild cognitive deficit, and, rarely, motor neuropathy. In a cohort of asymptomatic HTLV-1–infected individuals followed for up to 8 years, 30% developed neurologic signs or symptoms.[273] It is unclear whether these abnormalities indicate future progression to HAM or that HAM is simply the "tip of the iceberg" of a broader spectrum of neurologic manifestations associated with HTLV infection.[274]

Other Diseases Associated With HTLV-1

The infective dermatitis syndrome was first shown to be HTLV-1 associated in Jamaica.[275] It has also been seen in Trinidad and Tobago,[276] Colombia,[277] the Dominican Republic,[278] Brazil,[279] and central and southern Africa,[280,281] but very rarely in Japan, despite the high frequency of HTLV-1 carriers.[282] It seems to represent the first childhood HTLV-1 syndrome. Patients are born of HTLV-1–positive mothers and experience failure to thrive. They are prone to refractory generalized eczema and infection with *Staphylococcus* and *Streptococcus* bacteria that are suppressed by long-term antibiotic therapy and recur when the therapy is stopped. This syndrome usually emerges early in life, in the first few years after birth, and may persist into adulthood. Anecdotal cases emerging in adolescence suggest that some infective dermatitis cases may result from infection at an older age. It is postulated that infective dermatitis is an immunodeficiency syndrome induced by HTLV-1. Interesting to note, some patients go on to develop ATL and HAM.[282,283] Further study of the pathogenesis of this syndrome should provide valuable insights into the pathogenesis of HTLV-1–associated diseases.

Polymyositis, also known as HTLV-1–associated inflammatory myopathy of skeletal muscle, is rarely associated with HTLV-1 seropositivity in viral-endemic areas.[284] These cases are indistinguishable from polymyositis seen in HTLV-1–nonendemic areas. A large joint polyarthropathy has been reported in Japan among elderly patients.[285,286] A distinguishing feature of these cases is the presence of HTLV-1–producing cells in the synovial infiltrate.

A unique form of uveitis has been observed in HTLV-1–positive individuals in Japan and Australia.[287,288] The first evidence of an association of HTLV-1 infection with uveitis was reported by Ohba and colleagues in Japan, who detected ocular involvement in patients with ATL and HAM and asymptomatic carriers.[289] The ocular manifestations were then classified into three groups: (1) opportunistic infections and tumor infiltration in ATL patients; (2) ocular alterations in HAM patients, including Sjögren syndrome, retinal pigmentary degeneration, optic atrophy, vitreous opacities, cotton-wool spots, and retinal vasculitis; and (3) HTLV-1 uveitis in asymptomatic carriers. Proviral DNA of HTLV-1 was identified in 60% of T cells from intraocular fluid of these patients.[289,290]

HTLV-1–associated infiltrative pneumonitis has also been reported in some individuals in Japan.[291,292] Recently, HTLV-1 infection has been linked to increased occurrence of bronchiolitis, chronic bronchitis, and bronchiectasis in indigenous Australians.[293,294] The risk of pulmonary disease and bacteremia was associated with higher HTLV-1 proviral load in this HTLV-1c–endemic population. A Brazilian study using chest computed tomography (CT) found evidence of bronchiectasis and other structural abnormalities in patients with HAM but not asymptomatic HTLV-1 carriers.[295] The HAM patients also had more pulmonary function abnormalities, but cigarette smoking histories were not reported.

The association of HTLV-1 with parasitic infestations (e.g., *Strongyloides* or crusted scabies) refractory to treatment has also been interpreted to suggest that HTLV-1 may have immunosuppressive effects.[296,297] A variety of subclinical perturbations in hematologic markers, such as depressed hemoglobin and lymphopenia, have been reported in healthy HTLV-1 carriers.[298] In addition, a study showed that HTLV-1 subjects had higher platelet counts (+16,544 cells/mm^3) than seronegative subjects.[299]

Other possible consequences of HTLV-1 infection include persistent lymphadenopathy in offspring of HTLV-1–positive women (including HTLV-uninfected children)[155] and waxing and waning lymphadenopathy seen in adult carriers. This lymphadenopathy may be a manifestation of a primary or secondary immune response to HTLV infection. The influence of HTLV-1/HIV-1 on the progression to AIDS is controversial because soluble factors produced by HTLV-1–infected cells can either enhance or inhibit replication of HIV-1. The findings of several epidemiologic and in vitro studies have been mixed,[171,300,301–306] which suggests that differences in study outcomes may be due to phenotypic differences in HIV-1 clinical disease.[307] It is noteworthy that HTLV-1/HIV-1–coinfected persons have increased CD4$^+$ counts but still develop opportunistic infections.[308]

Diseases Associated With HTLV-2

Although the disease associations with HTLV-2 are less clear than those with HTLV-1, there is accumulating evidence showing a link to HAM and to other neurologic abnormalities.[9] Prospective studies of former blood donors who are HTLV-2 infected have shown that they are at increased risk for developing HAM,[309] although their incidence is lower than that reported for HTLV-1 carriers.[310] Among these infected individuals, HTLV-2 proviral loads appeared to be lower than HTLV-1 proviral loads, which may explain why HTLV-2 has been less associated with the development of disease.[311] There have been four cases of HTLV-2 HAM in the HTLV Outcomes Study,[218,312] and there are a number of HTLV-2 HAM case reports in the literature.[313–319] Sensory neuropathy has been observed with HTLV-2 alone[320] and with HIV coinfection.[320–322] In addition, a spinocerebellar syndrome has been documented in HTLV-2–infected patients.

HTLV-2 infection has also been diagnosed in a few cases of hematologic malignancies, including atypical hairy cell leukemia,[5,323] and with some cases of large granular lymphocytic leukemia[324] and mycosis fungoides.[325] However, systematic surveys have not identified a clear association of these lymphoid malignancies with HTLV-2, suggesting that these patients may be coincidentally infected. One retrospective study of HTLV-2–positive drug users showed an excess of asthma-related deaths and an increased frequency of skin and soft tissue infections.[326] In a prospective cohort study, HTLV-2 has been associated with pulmonary disease, arthritis, and urinary symptoms.[63] HTLV-2 subjects also had higher mean lymphocyte counts (+7%) and platelet counts over 14 years of follow-up.[299] Finally, HTLV-2 has been associated with increased overall mortality and cancer mortality.[327,328]

In contrast to the possible association between HTLV-1 and accelerated HIV-1/AIDS progression, some studies of HTLV-2/HIV-1 coinfected persons suggest a neutral or protective effect, especially among those with high HTLV-2 proviral loads, possibly as a result of chemokine overproduction.[329,330] Further studies are needed to confirm these findings.

THERAPY

Although first-generation nucleoside analogues, such as zidovudine and lamivudine, have been recognized to have activity against HTLV reverse transcription in vitro, there is little clinical evidence of their efficacy in vivo.[331] Research with raltegravir, an antiviral agent approved for treatment of HIV-1, has shown promise in reducing proviral load in vitro, but a small clinical trial showed no significant reductions in infected individuals.[332,333] However, until further research is completed, the asymptomatic nature of HTLV-1 and HTLV-2 and the low penetrance of HTLV diseases mean that treatment of asymptomatic HTLV carriers is not currently indicated. Furthermore, the exact role of HTLV-1 in disease pathogenesis has not been clearly defined. In ATL, active viral replication does not appear to play a role in established malignant disease,

and tumor cells harbor oncogenic mutations in cell-regulatory genes that may not be reversible by treating the virus. HAM, with its high viral load and substantial cell-mediated immune response to HTLV-1, would appear to be a better candidate for antiviral treatment. A combination of zidovudine and lamivudine was used in a clinical trial of HAM treatment, but no clinical improvement was seen and there was no effect on HTLV-1 proviral load or immunologic markers.[334] Therapy that targets the immune response itself may afford an equally attractive avenue for the experimental treatment of HAM. Because of the shared routes of exposure, there is a potential opportunity to investigate the impact of antiviral therapy on persons coinfected with HTLV-1 or HTLV-2 and HIV-1. In fact, HTLV-2 proviral load actually increased after highly active antiretroviral therapy in patients with HIV-1/HTLV-2 coinfection.[335] The increase in HTLV-2 proviral load appeared to correlate with the magnitude of $CD4^+$ lymphocyte proliferation after successful control of HIV viral load, which suggests a clonal rebound of HTLV-2–infected lymphocytes.

Asymptomatic HTLV-1 and HTLV-2 Carriers

Evaluation and treatment of asymptomatic HTLV-1 and HTLV-2 carriers are the most frequently encountered clinical situations and generally follow HTLV diagnostic screening of asymptomatic blood, tissue, sperm, or oocyte donors. The first step is to confirm HTLV infection, either by review of positive screening EIA and confirmatory tests performed by a reputable testing laboratory or by submission of another specimen. False-positive results are common when EIA is used alone in low-risk patients; these patients may be relieved of a false diagnosis by the simple performance of a confirmatory assay. Typing of the infection as HTLV-1 or HTLV-2 is important because of the different disease outcomes associated with the two viral types. This can be done through type-specific WB or line immunoassay, differential titration on an immunofluorescence assay, or PCR assay. A clinical history regarding risk factors for HTLV infection is important in establishing the pretest probability of infection and can be helpful in typing the infection. Familial or sexual contact with people from HTLV-1–endemic areas favors that infection, whereas a history of injection drug use or sex with an injection drug user is more consistent with HTLV-2 infection. As mentioned earlier, patients with indeterminate WB results are also unlikely to be infected if they have no risk factors for HTLV infection.

Asymptomatic seropositive patients should be followed by their primary care or infectious disease physician with annual to biannual return visits. Medical history should elicit symptoms of leukemia, lymphoma, or neurologic disease. Physical examination is directed at the skin, lymph nodes, and neurologic system to detect manifestations of HTLV dermatitis, ATL, or HAM. Laboratory evaluation may be limited to a complete blood and white blood cell differential count. Although increases in the absolute lymphocyte and platelet counts have been described in prospective studies of HTLV-1 and HTLV-2 carriers, there is no indication that these have clinical significance.[299] It is more important to rule out subclinical leukemia by means of a normal lymphocyte count and absence of flower cell morphology. In general, asymptomatic carriers or those with nonspecific symptoms should be reassured with reminders of the low penetrance of hematologic and neurologic disease. Attention should be devoted to counseling regarding the prevention of further HTLV transmission through the use of condoms and safe sexual practices and the avoidance of breastfeeding neonates born to HTLV-infected women (at least in developed countries). There is also substantial psychological and social morbidity associated with chronic HTLV infection, and the physician needs to probe for and address these issues.[336–338]

Adult T-Cell Leukemia

Treatment of ATL patients depends on the type and extent of disease and has evolved with the discovery that some types of disease are better managed with zidovudine and IFN-α instead of traditional lymphoma chemotherapy.[339] The lymphoma type of ATL is an aggressive high-grade lymphoma with a generally poor prognosis, although prolonged remissions are seen.[222,340] In Japan, large trials of vincristine, cyclophosphamide, prednisolone, and doxorubicin with the addition of methotrexate, and more complex 9- and 10-drug regimens, have shown some success without prolonging long-term survival.[340,341] Initial response rates, even for the poorest risk categories, are more than 50%, and complete remissions are achieved in 20% of patients, but these responses can be short-lived, with relapses occurring in weeks to months.[340] Poor prognostic factors include poor performance status at diagnosis, age older than 40 years, extensive disease, hypercalcemia, and high serum lactate dehydrogenase level. Thirteen percent to 15% of patients with such aggressive cases experience long-term survival (>2 years), which in one study was associated with several factors: complete remission, longer time to remission, and total doxorubicin dose. Relapses in these long-term survivors often occurred in the CNS and proved refractory to subsequent therapy. Studies using combinations of doxorubicin and etoposide have demonstrated complete remission rates of 40%.

Substantial improvements in therapy for other ATL types (i.e., smoldering, chronic, and leukemia-type) have been achieved with newer regimens combining zidovudine and IFN-α.[342] Although its mechanism of action is not well defined, this combination produces a high rate of complete responses and prolongs survival.[343–346] Measurement of HTLV reverse transcriptase activity has suggested that ongoing HTLV replication might promote ATL and explain the mechanism of zidovudine and IFN-α action.[347] A meta-analysis has demonstrated superiority of this regimen for all ATL types except the lymphoma type.[348]

Therapy approaches for high-grade lymphomas including allogeneic bone marrow transplantation and autologous stem cell transplantation have resulted in remission and should be strongly considered in younger patients.[349] One case showed reappearance of cells harboring the integration of HTLV-1 previously observed in his leukemia cells, but the patient continued in clinical remission, suggesting a possible reversion to the preleukemic carrier state.[350] Experimental approaches that use arsenic trioxide or monoclonal antibodies targeting the IL-2R specific to ATL cells are being tested, with some evidence of at least partial responses.[224,351–353] Current trials for ATL include ruxolitinib, a Janus kinase 1 and 2 inhibitor, and for relapsed or refractory lymphoma include subcutaneous recombinant human IL-15 plus alemtuzumab, and nivolumab (www.ClinicalTrials.gov). A consensus report on ATL treatment has been published recently.[353a]

HTLV-1–Associated Myelopathy

Advances in the treatment of HAM have been hindered by the difficulty of performing randomized clinical trials because most cases occur in resource-limited countries. Accurate measurement of disease severity has also been a challenge in such trials, but the Osame disease severity scale[354] provides clinical staging and the 10-meter and 6-minute walk tests may be more quantitative.[355] Traditionally, immunosuppressive therapy with corticosteroids,[256,356] cyclophosphamide,[357] or both has been used to some benefit, particularly in acutely progressive cases. Toxicity limits the long-term use of these drugs, but short-term pulse therapy with intravenous methylprednisone may be of benefit for pain if not functional capacity.[358] More recently, IFN-α and IFN-β1a have shown some clinical benefit but fall short of definitive treatment.[359–361] Danazol, an androgenic steroid, has been used for symptomatic treatment of bladder and bowel symptoms resulting from spinal cord involvement in HAM, but it does not reverse the underlying neurologic deficit. The heparinoid pentosan polysulfate and the thiamine derivative prosultiamine were shown to improve motor function in some patients in small, open-label trials.[362,363] Given the emerging picture of disease pathogenesis with the inability to control high viral expression, therapy with antiviral drugs would seem a promising avenue for research, but initial clinical trials of antiretroviral therapy have not been successful.[334] Globally, current clinical trials for the treatment of HAM/TSP include mogamulizumab—a humanized antibody engineered to promote cellular cytotoxicity—raltegravir, and natural killer (NK) cell immunotherapy. Other studies underway are supportive and adaptive in nature, including sensorimotor exercises using a virtual reality platform, and urologic physical therapy for those with urinary symptoms. Recently completed trials include Mik-Beta-1 monoclonal antibody (Hu Mik-Beta-1), infliximab, anti-Tac antibodies directed against CD25 IL-2R α-chain

concurrently with zidovudine, pentoxifylline, zidovudine plus lamivudine, cyclosporine, and bortezomib plus raltegravir (www.ClinicalTrials.gov). The International Retrovirology Association publishes current treatment recommendations on its website (www.htlv.net/HAMpdf).

PREVENTION

Guidelines for HTLV-1 and HTLV-2 prevention and counseling were developed in 1993 by a Centers for Disease Control and Prevention working group; an update of these guidelines to incorporate more recent evidence would be welcome.[364] Standard prevention approaches address each of the routes of transmission and are similar for both viruses: screen blood donations before transfusion, eliminate breastfeeding by known infected mothers, and advise the use of condoms.

The value of blood donor screening has been well documented in highly endemic regions of Japan. In areas where the infection is not endemic, such as the United States, the cost-effectiveness of such screening has been questioned, but current FDA guidelines recommend HTLV testing of all blood donors but not currently of organ donors.

In high-income countries, it is recommended that pregnant women who are HTLV-1 positive should not breastfeed their infants. In developing countries where safe alternatives to breastfeeding may not be available, limiting breastfeeding to the first 3 months may afford some protection because of passively transferred maternal antibodies, although the safety of this approach has been cast in doubt.[161] The use of condoms is recommended for sexually active HTLV seropositive individuals and for couples who are serodiscordant for HTLV infection. Given the relatively low frequency of sexual transmission for each sexual encounter, couples who desire a pregnancy could plan to have unprotected sex during periods of maximal fertility. Techniques such as sperm washing, developed to allow assisted reproduction for HIV-infected persons, may also be used in the HTLV setting.

Population-based screening for HTLV infection (except for blood donors) is generally not indicated because of the low penetrance of disease and lack of effective therapies. The one exception in some countries may be HTLV-1 screening of pregnant women followed by interdiction of breastfeeding; such screening has been shown to be cost-effective in the United Kingdom.[365] Counseling seropositive patients should involve a clear discussion of the distinction of HTLV from HIV. In addition, HTLV type should be defined by serologic methods, and the distinctions in disease associations of the two virus types should be emphasized. Because the populations at risk for HIV are also at risk for HTLV-1 infection in viral endemic areas (e.g., persons at risk for sexually transmitted diseases, persons with high rates of partner exchange, commercial sex workers), HIV prevention guidelines also benefit those at risk for infection with HTLV-1. Thus, prevention measures that promote condom use, treatment of sexually transmitted infections, and decrease of high-risk exposures also prevent HTLV-1 infection.

There is no therapy for HTLV-1 infection and thus no chemoprophylaxis. Passive immunoprophylaxis is hypothetically effective, as noted earlier in animal studies, but has no practical clinical application, given the low risk for transmission, except through sexual contact, breastfeeding, and transfusion exposure, wherein other prevention methods are more applicable.

Although vaccines against HTLV-1 are potentially feasible, owing to the low penetrance of disease there has been little impetus to develop or market an HTLV-1 vaccine. Experimentally, vaccines containing whole-virus and recombinant HTLV-1 envelope antigens have successfully prevented HTLV-1 infection in monkey and rabbit models.[366] Protection correlates with the presence of neutralizing antibodies, indicating that humoral immunity can be an effective barrier against infection even when the challenge is cell associated.[202,367] The HTLV-1 envelope is relatively highly conserved, and neutralizing antibody appears to protect against challenge with even major strain variants, consistent with the conclusion that a single serotype protects against all variants. Thus, a synthetic vaccine against one HTLV-1 isolate could protect against other HTLV-1 isolates. A vaccine that induces cell-mediated immune responses in nonhuman primate studies has also been shown to be effective.

CONCLUSION

The study of HTLV-1 and HTLV-2 is important for public health, clinical, and scientific reasons. In the years since the discovery of this first human leukemia virus, significant progress has been made in understanding the biology and epidemiology of HTLV. Routes of transmission for HTLV-1 and HTLV-2 are well defined, and sexual and parenteral transmission may be controlled through appropriate public health interventions, including those directed at the HIV epidemic. Knowledge of the clinical outcomes of HTLV infection has expanded since the discoveries of the relationships between HTLV-1 and ATL and between HTLV-1 and HTLV-2 and HAM. A growing array of syndromes related to the virologic or immunologic effects of these retroviruses has been recognized, and prospective observational cohorts of persons infected with HTLV-1 and HTLV-2 will yield additional data on their clinical outcomes. Among those infected with HTLV-1, the incidence of either ATL or HAM is less than 5%, although there is substantial mortality in the former and morbidity in the latter. With millions of people estimated to be infected by HTLV-1 worldwide[73] and a lack of effective therapy,[368] prevention efforts are important. Blood transfusions need to be screened, sexual transmission prevented with condom use, and breastfeeding by HTLV-infected mothers limited, although the last approach may not be feasible in developing countries. In the United States, public health guidelines for the control and management of HTLV-1 and HTLV-2 infections are outdated and need to be revised.

From a scientific viewpoint, the HTLVs offer interesting models for understanding the interplay between chronic retroviral infection and the human immune system that determines the latent proviral reservoir and potential approaches for its eradication and cure of HTLV and HIV infections. Understanding the virology, immunology, and pathogenesis of HTLV infection may be relevant to the study of leukemia, lymphoma, and multiple sclerosis. It is also conceivable that additional viruses of this class with long latency, low-level replication, and specific cellular tropism may be discovered in unexplained autoimmune, neurologic, and malignant diseases.

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169 Human Immunodeficiency Viruses

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SHORT VIEW SUMMARY

Definition
- Human immunodeficiency virus (HIV) is a *Lentivirus*, family Retroviridae, that causes acquired immunodeficiency syndrome (AIDS). There are two types, HIV-1 and HIV-2, of which HIV-1 is the more widely distributed and more pathogenic.

Epidemiology
- HIV infection is worldwide in distribution and is transmitted by sexual activity and intravenous drug use.

Microbiology
- HIV is a single-stranded positive-sense RNA virus that contains three structural genes *(gag, pol, env)* and six regulatory genes. HIV infects $CD4^+$ T cells and uses its reverse transcriptase (RT) to transcribe its RNA genome into double-stranded DNA, which is integrated into the host cell genome.

Diagnosis
- Infection is identified by detection of viral RNA (virus load) or by immunologic responses to viral proteins (Western blot) (see Chapter 120).

Therapy and Prevention
- Antiretroviral therapy (ART) has been developed using nucleoside and nonnucleoside RT inhibitors, protease inhibitors, integrase inhibitors, and entry inhibitors (see Chapter 128). ART can also decrease the rate of transmission of infection. Vaccine development seeks to stimulate immune responses to HIV antigens that will inhibit transmission and/or replication of virus (see Chapter 119).

OVERVIEW OF HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME

Infection with human immunodeficiency virus type 1 (HIV-1) and its end stage, acquired immunodeficiency syndrome (AIDS), is one of the major public health challenge of modern times, with more than 25 million persons already dead and 30 to 40 million living with HIV/AIDS, many of whom are without access to therapy. AIDS was first recognized in the United States in 1981 with reports of unexplained opportunistic infections, including *Pneumocystis jirovecii* pneumonia and Kaposi sarcoma (KS), among homosexual men in New York and San Francisco.[1–3] On the basis of the epidemiologic features, association with the loss of $CD4^+$ lymphocytes and immunosuppression, and likely infectious cause, a new human retrovirus was postulated as a causal agent. The field of retrovirology had markedly advanced just a decade earlier with the description of reverse transcriptase (RT) and with the discovery of human T-cell lymphotropic virus types 1 and 2 (HTLV-1 and HTLV-2), the first two known human retroviruses, in 1979 and 1981 (reported in 1980 and 1982, respectively).[4,5] The discovery of interleukin-2 (IL-2), or T-cell growth factor,[6,7] allowed the culture of blood T lymphocytes from early cases of AIDS; by 1984 the detection, isolation, and propagation of HIV-1, the third human retrovirus,[8–12] had led to the development of an immunodiagnostic test, an increasingly detailed understanding of the molecular biology of this virus, and, most important, the introduction of a rational basis for antiviral therapy.[13] Eventually new therapeutic combinations (RT and protease inhibitors), combined with the ability to measure circulating viral RNA and resistance to drugs, led to a dramatically improved clinical course for those with access to therapy. More recently, inhibitors of virus-cell fusion, viral entry into the host cell, and integration of viral DNA into host chromosomal DNA have become available. Within a brief period, technologic advances provided a clearer understanding of viral dynamics and the disease process, focusing attention on viral replication, host immune responses, and T-cell dynamics while confirming and elaborating the causal role of the virus. Equally dramatic has been the elucidation of how HIV enters cells, using both the CD4 molecule and a chemokine receptor as a dual-receptor system, as well as the mapping of the three-dimensional structure of the viral envelope protein. Contemporary retrovirology is largely devoted to the study of HIV-1 and of HIV-associated diseases. The molecular and cellular biology of this virus is now better understood than that of almost any other in history. Research on HIV showed that rational antiviral therapy is possible and thereby pointed the way toward therapy for other viral diseases. Although this information has yet to be completely translated into greater progress in the areas of prevention, therapy, vaccines, and immune reconstitution for much of the developing world, where most HIV is transmitted, the Presidential Emergency Plan for AIDS Relief (PEPFAR) and other programs have greatly facilitated the institution of effective therapies in many parts of Africa.

Viruses are obligate intracellular parasites, and every aspect of the virus is in some way relevant to virus-host relationships. This chapter outlines the life cycle, molecular and cellular biology, structure, and regulation of HIV-1 and includes some discussion of pathogenesis and outcomes of infection. Although the division of the chapter into sections is useful for organization of the information presented, in reality these subjects cannot be separated from one another; from the perspective of both virus and host, the process of infection is a continuous series of connected interactions.

ORIGIN AND CLASSIFICATION OF HUMAN RETROVIRUSES

Current knowledge has placed retroviral infection of humans as zoonoses that originated in primate-to-human species-jumping events. For HIV-1 and HIV-2, these events occurred in Central and West Africa, most likely at multiple times, with the more recent attaining major epidemic significance. Simian immunodeficiency virus of chimpanzees (SIV_{cpz}) is the immediate precursor to HIV-1.[14] It is likely that similar species-jumping events occurred previously between certain types of monkeys and chimpanzees.[15]

Retroviruses are classified by a number of different biologic features into at least seven genera. Oncogenic retroviruses occur in all classes of vertebrates. The first identified infectious agents that produced cancer in chickens were isolated by Ellerman and Bang (1908)[16] and by Peyton Rous (1910).[17] These workers were considerably ahead of their time, and biologic systems to culture and study these viruses had not yet been described. Rous eventually won a Nobel Prize in 1966 for his work. The pioneering work of Ludwig Gross in the 1950s stimulated

renewed interest by demonstrating that oncogenic viruses could produce tumors in mammals,[18] but for the next 3 decades most scientists believed that human retroviruses did not exist. We now know that the pathogenic human retroviruses include lentiviruses (HIV-1 and HIV-2) and oncoviruses (HTLV-1 and HTLV-2). A variety of human endogenous retroviruses, present in the human germline, are often, but not always, replication defective and have not yet been shown to cause any disease.

As their major replication strategy, retroviruses reverse transcribe viral RNA into linear double-stranded DNA (dsDNA), with subsequent integration into the host genome. The characteristic enzyme used for this process, an RNA-dependent DNA polymerase that reverses the flow of genetic information, is known as RT. Its discovery altered the "central dogma" of molecular biology—namely, that genetic information only flows from DNA to RNA[19,20]—and helped initiate the modern era of molecular biology. RT is error prone; with the massive turnover of virions in the infected host, these errors accumulate in the viral DNA, accounting for the high mutability of HIV-1. The lifestyle of the retrovirus thus involves two genomic forms, a DNA provirus and an RNA-containing infectious virion.

The discovery of HTLV-1 and its etiologic association, first with adult T-cell leukemia, an aggressive T-cell lymphoma,[21-23] and later with a neurologic disease, tropical spastic paraparesis/HTLV-1–associated myelopathy (TSP/HAM),[24] were pivotal events in modern medicine (see Chapter 168). In an absolute sense, HTLV-1 is not very efficient in inducing lymphoma (~10% lifetime risk), but it is quite efficient compared with other human carcinogens, including other viruses. Although there is relatively little variation among HTLV-1 isolates, HTLV-2, the second human retrovirus, is only 50% identical to HTLV-1 at the genomic level.[4] Similarly, HIV-2, the fourth human retrovirus, was identified as a serologic variant of HIV-1, the third human retrovirus, and was isolated from patients in western Africa.[25,26] Some types of SIV are so closely related to HIV-2 that they may form an overlapping continuum with recent common ancestors. HIV-2 is known to infect several monkey species, including the sooty mangabey, its original host, and SIV has been transmitted, albeit rarely, to laboratory workers. SIVs and SIV/HIV hybrids (SHIVs) have been used extensively to study animal models of immunodeficiency. Other species, including cats (feline leukemia virus [FeLV] and feline immunodeficiency virus [FIV]) and cattle (bovine leukemia virus [BLV] and bovine immunodeficiency virus [BIV]), harbor retroviruses analogous to those of humans and some African primates. HIV-related retroviruses, known as lentiretroviruses, also include the ungulate viruses, maedi-visna virus of sheep, caprine arthritis-encephalitis virus, and equine infectious anemia virus.

As RNA viruses, retroviruses have the survival advantage of great genetic diversity. As viruses with a DNA intermediate in their replication cycle, they also have the advantage of latency, as do many DNA viruses, but even more so because the DNA provirus is integrated into the chromosomal DNA of the infected cell. As a $CD4^+$ T-cell and macrophage-tropic virus, HIV also has the advantage of reducing the effectiveness of the host immune response.

Retroviruses are typically approximately 100 nm in diameter and contain two single strands of RNA, which permits recombination between the strands (Fig. 169.1). The typical genome is approximately 10 kilobases (kb) or less in size and contains three major structural genes—namely, *gag* (group-specific antigen; core structural proteins), *pol* (polymerase; RT and other enzymes), and *env* (envelope proteins). HIV-1 also contains several additional genes; similar "extra" genes were first described in HTLV-1. More recently, antisense transcripts and proteins, of unclear function, have been described for both HIV-1 and HTLV-1.[27,28] In both viruses some of the extra genes are essential to viral replication, whereas others may modulate interactions of the virus with its host. Fig. 169.2 outlines the genome composition of HIV-1 and HTLV-1.

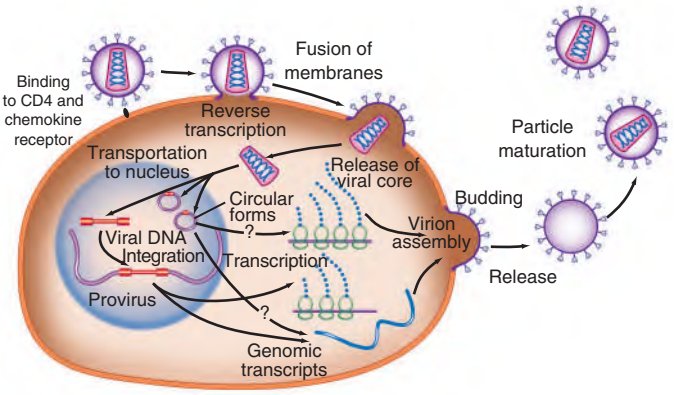

FIG. 169.1 The life cycle of human immunodeficiency virus type 1.

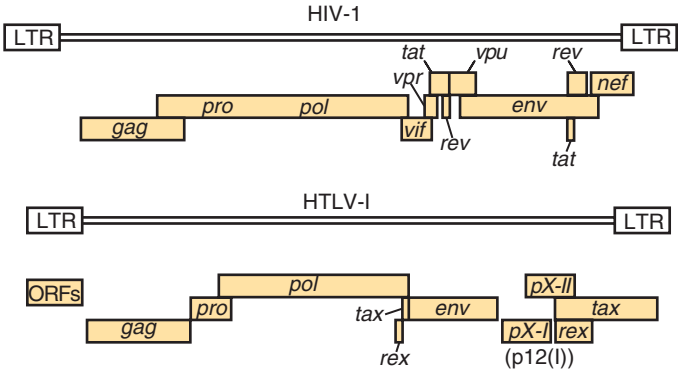

FIG. 169.2 Genomic organization of human retroviruses. A comparison of the genomes of human T-cell lymphotropic virus type 1 *(HTLV-1)* and human immunodeficiency virus *(HIV)* is shown. Studies of HTLV genes and gene products laid the foundation for an understanding of their functional homologues subsequently found in HIV (e.g., *tat* and *rev*), although there is little sequence homology of these genes between HTLV and HIV. *env,* Envelope; *gag,* group-specific antigen; *LTR,* long terminal repeat; *nef,* negative regulatory factor; *ORFs,* open reading frames; *pol,* polymerase; *pro,* protease; *rev,* regulator of viral expression; *rex,* regulator of viral expression; *tat,* trans-activator; *tax,* trans-activator of transcription; *vif,* virion infectivity factor; *vpr,* viral protein R; *vpu,* viral protein U.

VIRAL TRANSMISSION AND LIFE CYCLE

Biology of Transmission

The infectious life cycle of HIV can be described both at the molecular, single-cell level and at the level of a host organism infected with a "swarm" of closely related viral species. Multiple variables, including route of exposure, dose, immunogenetic background, and concomitant infections, influence the probability of transmission. The biologic events during exposure and successful infection of the host are only partially elucidated. The most common modes of infection are sexual transmission at the genital or colonic mucosa; exposure to other infected fluids, such as blood or blood products; transmission from mother to infant; and occasionally accidental occupational exposure, such as needlesticks. Transmitted viruses typically use the interaction of the viral glycoprotein gp120 with the cellular receptor CD4 and the chemokine receptor CCR5 to gain cell entry, thus selecting for macrophage-tropic *non–syncytia-forming* variants. This finding explains why persons who lack a functional CCR5 receptor are relatively resistant to infection by sexual transmission. Non–macrophage-tropic strains, also called *syncytia-inducing*, are typically found late in infection. These strains use another chemokine receptor, CXCR4, to facilitate entry and are apparently not readily transmitted from person to person. In a model of acute infection in the macaque, the first cellular targets of intravaginal inoculation of the virus are Langerhans cells, tissue dendritic cells in the lamina propria that then fuse with lymphocytes. However, direct infection of T cells has also been described. Infected cells can be found in draining lymph nodes within 2 days and in plasma after 5 days.[29]

In HIV-1–infected persons, there is a rapid rise in plasma viremia within days, with high viral titers and widespread dissemination, probably targeting lymphoid organs and the central nervous system (CNS). This acute stage of HIV infection is sometimes manifested as a transient

symptomatic illness characterized by a maculopapular rash and flulike symptoms. This phase is followed by a marked reduction in virus to steady-state levels, probably because of vigorous antivirus cellular responses. The immune response probably accounts for the mononucleosis-like acute syndrome seen in approximately half of patients. Initially, perhaps within hours of infection, at least three HIV inhibitory chemokines (notably macrophage inflammatory protein-1α [MIP-1α], MIP-1β, and RANTES [regulated on activation, normal T-cell expressed and secreted]) may be produced. As is the case for many infections with viruses that establish chronic infections, this response is at least partially successful in controlling replication. Levels of HIV-1–specific cytotoxic T lymphocytes (CTLs) are inversely correlated with plasma viral RNA levels.[30] High levels of potent CTL virus-specific cells targeted to the viral Env protein and soluble factors produced by these cells early in infection may correlate with the decline of virus, even before a neutralizing antibody can be detected.[31]

Great variability in peak viral RNA plasma levels is seen during the first 120 days after infection. By 3 to 6 months after infection, viral levels reach a temporary steady state, sometimes called a viral set point. This level positively correlates with subsequent disease progression. Thus, early in the course of infection, virus-host interactions are established that are predictive of subsequent disease.[32]

Intervention to control infection during this initial period has been shown to decrease the risk of subsequent infection in health care workers after needlestick exposure.[33] Treatment of infected mothers and exposed neonates has also shown a dramatic effect in decreasing the incidence of maternal-fetal transmission.[34] Likewise, treatment with antiretrovirals, such as Truvada, greatly reduce sexual transmission of HIV-1,[35] although drug-resistant variants may still be transmitted.

Replication Cycle: Cell Entry and Integration

The replication cycle of HIV-1 can be considered in two distinct phases (see Fig. 169.1). The first phase includes viral attachment, entry into the cytoplasm, reverse transcription, entry into the nucleus, and integration of the dsDNA (the provirus). The second phase occurs over the lifetime of the infected cell as viral and cellular proteins regulate the production of viral proteins and new infectious virions.

Infection is initiated by the binding of the virion gp120 Env surface protein to CD4, found on some T cells, macrophages, and microglial cells. Both SIV and HIV-2 also use CD4. CD4 was first identified as a viral receptor in a number of studies showing the susceptibility of CD4-bearing cells to infection and the ability to block infection with anti-CD4 monoclonal antibodies in culture. Transfection of human $CD4^-$ HeLa cells with CD4 DNA rendered them permissive for infection.[36] Successful in vitro experiments blocking this interaction with soluble CD4 used laboratory strains adapted to cell lines and led to therapeutic attempts using immunoglobulin CD4, which were unsuccessful. Subsequent experiments showed that primary isolates are insensitive to soluble CD4 and highlighted the necessity of using primary rather than laboratory-adapted isolates in studying virus-host interactions.

As with other lentiretroviruses, macrophages could also be infected with HIV, but strains differed in their ability to infect T-cell lines or monocytes.[37] Binding to CD4 is not sufficient for cellular entry of HIV, and the fact that small changes in the V3 loop of envelope gp120 (see later) could determine tropism of the virus for either macrophage or T-cell lines suggested that a second receptor was present. The first important clue for the basis of this tropism was the unexpected finding that a group of chemokines (RANTES, MIP-1α, and MIP-1β), small extracellular proteins naturally produced by $CD8^+$ T cells, inhibited macrophage-tropic but not T-cell line–adapted strains.[38] This discovery at once explained the nature of a long-sought CD8 viral suppressor factor and suggested a previously unexpected role for chemokine receptors. The independent identification of an orphan chemokine receptor, CXCR4, as the second receptor for T-cell line–tropic strains[39] was followed by a rapid series of reports demonstrating CCR5 to be the principal second receptor for macrophage-tropic strains and CXCR4 for T-cell line–adapted strains.[40–43] Crystallographic evidence indicates that CD4 binds in a recessed pocket on gp120, which includes a deep cavity that binds to phenylalanine-43 of CD4. Previous mutagenesis studies had shown this phenylalanine to be crucial for binding.[44] Other studies have also indicated a role for sugar molecules, glycosaminoglycans, in the binding of gp120, which influences interactions with the chemokine receptor.[45] CD4 binding triggers a conformational change in gp120 that allows it to then bind to CCR5 or CXCR4. This second binding event exposes the fusion domain of the gp41 transmembrane protein, allowing fusion of the viral and cellular membranes, followed by viral entry into the target cell.

Events that occur immediately after viral entry—collectively, the disassembly process—are highly regulated. For example, HIV-1 must incorporate a cellular protein, cyclophilin A, which binds to the viral capsid protein p24. Failure to incorporate this cellular protein results in a profound postentry block during the next viral entry. Coincidentally, cyclophilin (peptidyl-prolyl isomerase) is the binding protein for cyclosporine, an inhibitor of T-cell activation, suggesting that activation-related cellular processes are involved in viral disassembly.[46,47] In addition, virion infectivity factor (Vif) and "negative" regulatory factor (Nef; a misnomer), accessory viral proteins, may also be required.[48]

Reverse transcription begins in the cytoplasm, as DNA synthesis is initiated from a cellular transfer RNA (tRNA) primer bound to the viral genomic RNA just downstream of the 5′ long terminal repeat (LTR). Reverse transcription proceeds in a similar orderly fashion in all retroviruses. Briefly, the transcription complex begins at the 5′ end, copies the U5 and R regions of the 5′ LTR, and then jumps to the 3′ end of the RNA, where the newly synthesized R region DNA binds to the R region of the 3′ LTR. Reverse transcription continues through the U3 region of the 3′ LTR and then through the remainder of the viral RNA, which gives a complete minus strand of DNA. The RNA is degraded by the viral ribonuclease H, except for two resistant purine-rich tracts in the middle and toward the 3′ end of the viral RNA. These then serve as the primers for formation of the DNA plus strand.[49] Because reverse transcription takes place in the cytoplasm, local concentrations of nucleotides may be a limiting factor, particularly in nondividing cells. The cellular protein sterile alpha motif and HD-domain–containing protein 1 (SamHD1) dephosphorylates nucleotide triphosphates, reducing the precursor pool and inhibiting reverse transcription. SamHD1 is targeted for degradation by viral protein X (Vpx) of HIV-2 and some SIVs, but HIV-1 lacks Vpx and therefore has a reduced ability to infect dendritic cells, macrophages, and quiescent T cells.[50–52]

During the formation of dsDNA, the uncoated nucleoprotein complex, termed the *preintegration complex*, is imported into the nucleus. This is an energy-requiring process that uses nuclear localization signals present on viral Gag, viral protein R (Vpr), and integrase (IN) proteins. Unlike most retroviruses, which integrate into the host cellular DNA as the nuclear membrane is disrupted during cell division, HIV-1 can enter the nucleus and integrate into nondividing cells. This may be critical in the infection of monocytes and macrophages, which are essentially nondividing cells.

IN-negative mutants of HIV do not integrate and do not produce infectious virus.[53,54] Although integration is not site directed, HIV preferentially integrates into or near active genes, particularly those that are activated after infection by HIV-1.[55] Unintegrated viral DNA may survive, however, particularly in quiescent cells. This may provide a stable intermediate form in cells that are temporarily not permissive for infection; if cell activation occurs when these forms are present, viral infection may then proceed to completion. Integration of viral DNA establishes a linear copy of the viral genome within the genome of the cell, and replication of the virus then occurs with cell replication. Integration is generally for the life of the cell and, with the cell and its progeny, for the life of the organism.

Replication Cycle: Virus Expression and Production of New Virions

Synthesis of new viral RNA genomes and proteins is accomplished in a highly regulated manner by using host cell proteins. A high level of viral production from several different cellular compartments can be maintained throughout the course of infection. The high number of replication cycles allow the generation of variants and selection by drugs or the immune system. The half-life of virus-producing $CD4^+$ T cells is approximately 0.7 days, and the generation time of HIV-1 in vivo is

approximately 2 days.[56] HIV-1 pathogenesis is the result of a complex interplay between the virus and the immune system, particularly the mechanisms responsible for T-cell homeostasis and regeneration. Protracted loss of $CD4^+$ T cells results from early viral destruction of selected memory T-cell populations, followed by a combination of profound increases in overall memory T-cell turnover, damage to the thymus and other lymphoid tissues, and physiologic limitations in peripheral $CD4^+$ T-cell renewal.[57] Equally important are indirect hematopoietic and immunoregulatory effects of viral components (see later).

Once integration has occurred, virus production depends on the presence of cellular and viral factors required for activation of viral promoters. External factors, including coinfection with other agents, production of inflammatory cytokines, and cellular activation, may enhance viral replication.[58] The molecular mechanisms regulating virus production include cellular pathways involving factors, such as the nuclear factor kappa B (NF-κB) family of inducible transcription factors, that result in a cascade of events leading to viral genome expression.[59]

An important feature of HIV-1 is that expression of different viral RNA species is temporally regulated. Using cellular enzymes, such as RNA polymerase II, transcription of the provirus is initiated at the viral promoter, at the junction of the U3-R regions in the LTR, as a single complete message. The viral messenger RNA (mRNA) and genomic RNA transcripts, processed by cellular machinery, are spliced, capped, polyadenylated, and transported to the cytoplasm for translation into viral proteins. Differential splicing of this complete RNA, controlled in part by the viral protein Rev, determines the type of message and protein that is produced. Early after infection, activated cells produce 2-kb mRNAs for viral regulatory proteins that can be detected by Northern blot analysis[60]; using even more sensitive reverse-transcriptase polymerase chain reaction techniques, expression can be detected within 6 hours.[61] These messages include the unique double-spliced RNA for Tat, Rev, and Nef proteins.[62] Tat protein induces a markedly enhanced activity of the viral promoter, chiefly by facilitating the elongation of nascent short viral transcripts, resulting in greatly increased RNA and protein production. The Rev protein serves to decrease the production of double-spliced messages. With the accumulation of Rev protein, there is a switch to enhanced expression of unspliced and single-spliced mRNAs that encode the late viral proteins, including the virion proteins Gag, Pol, and Env and viral proteins Vpu, Vpr, and Vif, as well as genomic RNA. The delayed transit from early to late viral genes probably reflects a requirement for threshold levels of Rev needed to bind and form multimers of the protein complexed with the Rev regulatory element (RRE), located in incompletely spliced mRNAs. Packaging of the genomic RNA within a virus particle requires the presence of a specific packaging signal located between the major splice donor at the 5′ end of the genome and the initiation codon for the Gag precursor polyprotein. In the absence of this signal, mature particles are formed that are devoid of RNA. The incorporation of genomic RNA requires two zinc-finger domains found in the p7 Gag nucleocapsid protein. Assembly of mature viral particles occurs at the cell membrane with the association of the Gag matrix protein p17 with the cytoplasmic domain of the envelope transmembrane protein gp41, which in turn binds to the viral gp120 on the outer surface. Assembled virions include the viral envelope proteins, cell membrane and associated cellular proteins, a matrix composed mainly of Gag p17, and a capsid composed of the p24 capsid protein and RNA, RT, IN, Gag proteins p6 and p7, and Vpr. The mature viral particle characteristically buds from the cell surface into the surrounding media, completing the life cycle of the virus. Budding of HIV-1 virions occurs at highly specialized membrane microdomains known as lipid rafts.[63] These domains are characterized by a distinct lipid composition that includes high concentrations of cholesterol, sphingolipids, and glycolipids.

THE VIRION

The mature infectious virus particle buds from a cell membrane, forming a sphere with an outer lipid bilayer and a nucleocapsid with a dense, cone-shaped core (Fig. 169.3). The core appears to be attached to the viral outer envelope at its narrow end.[64] The outer membrane contains up to 72 spiked knobs, which are assembled as trimers of the outer envelope protein gp120 bound to the transmembrane portion gp41. The viral membrane is cholesterol rich and includes cellular proteins.[65]

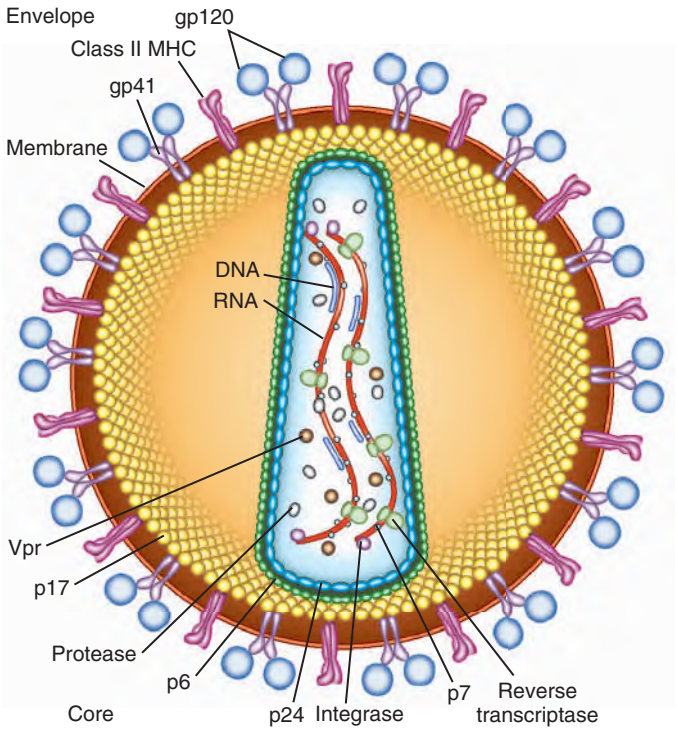

FIG. 169.3 Structure of the human immunodeficiency virus type 1 (HIV-1) virion. The viral envelope is formed from the host cell membrane, into which the HIV-1 envelope proteins gp41 and gp120 have been inserted, and may include several host cell proteins, most significantly the class II major histocompatibility complex *(MHC)* proteins. The matrix between the envelope and the core is formed predominantly from group-specific antigen (Gag) protein p17. The core contains the viral RNA, closely associated with Gag protein p7, in addition to reverse transcriptase (RT) and integrase. It has also been shown that virions contain complementary DNA, as shown, synthesized by the RT. The major structural proteins of the core are Gag proteins p24 and p6. Also present within the virion are the protease and two cleavage products from the Gag precursor protein (p1 and p2, not shown) of undetermined position within the virion. Viral protein R *(Vpr)* is also packaged in the virion and is thought to be localized within the core, as shown.

Each mature virion contains two molecules of single-stranded RNA surrounded by a capsid containing three *gag* gene cleavage products: the p17 matrix protein; the p24 major capsid protein, which forms the capsid shell; and the p7 nucleoprotein, which binds tightly to the viral RNA. The matrix contains the myristoylated matrix protein p17, which is critical for virion formation and is localized between the capsid protein p24 of the viral core and envelope. The p7 protein binds the two positive-strand copies of complete viral RNA attached at the packaging site, and it also binds to p24. Other viral proteins required for the early phases of infection are incorporated with the virion and include protease, which is essential for viral assembly; RT and IN, which are needed after entry for viral DNA synthesis and integration; $tRNA^{lys}$ at the 5′ end of the RNA, which serves as the primer for initiation of negative-strand DNA synthesis; and Vpr, a small protein that contains a nuclear localization signal and is associated with the nucleocapsid in large quantities. See Fig. 169.4 for a schematic view of the preintegration complex. The virus encodes at least five other regulatory and/or accessory genes of diverse function. Some have been discussed already, and most are present in the infected cell but not in the mature virion. A list of HIV genes and associated proteins is presented in Table 169.1.

Genomic Organization

The HIV-1 proviral DNA integrated into the host cell is 9.7 kb in length and follows the basic genomic structure common to most retroviruses:

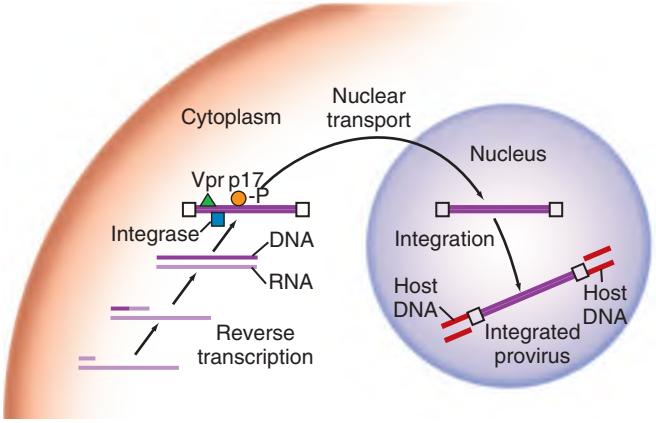

FIG. 169.4 Viral proteins required for nuclear transport and integration. After entry, formation of the reverse transcription complex and reverse transcription, human immunodeficiency virus (HIV) infection involves the formation of the preintegration complex, consisting of the newly synthesized viral DNA and several HIV proteins: the matrix protein *(p17),* integrase, reverse transcriptase, and viral protein R *(Vpr).*

TABLE 169.1 Genes and Gene Products of HIV-1 and HIV-2

GENE	PROTEINS	SIZE (kDa)	FUNCTION/PROPERTIES
gag	p17 p24 p6 p7 P1 p2		Matrix protein; interacts with gp41 Core protein Core protein; binds to Vpr Nucleocapsid; binds to RNA
pol	Protease Reverse transcriptase Integrase	10 66, 51 32	Proteolytic cleavage of Gag and Pol Polymerase and RNase H activity (p66 only) Integration into chromosome
env	gp120 gp41		Envelope; viral entry into cell Transmembrane protein; cell fusion
vif	Virion infectivity protein	23	Efficient cell-free transmission
vpr	Viral protein R	18	Enhances viral replication in primary cells, virion-associated protein; G_2/M phase arrest; nuclear localization
tat	Trans-activator of transcription	14	Major viral trans-activator, immune suppression
rev	Regulator of expression of virion protein	19	Enhances expression of unspliced and single-spliced RNAs
vpu[a]	Viral protein U	15–16	Enhances virion release from cells; downregulates CD4 and class I MHC surface expression
nef	Negative regulatory factor	27	Inhibits or enhances viral replication depending on strain and cell type Downregulates CD4; class I MHC Antiapoptosis
vpx[b]	Virion protein X	25	Packaged into the virion

[a]HIV-1 only.
[b]HIV-2 only.
HIV, Human immunodeficiency virus; *kDa,* kilodaltons; *MHC,* major histocompatibility complex; *RNA,* ribonucleic acid; *RNase H,* ribonuclease H.

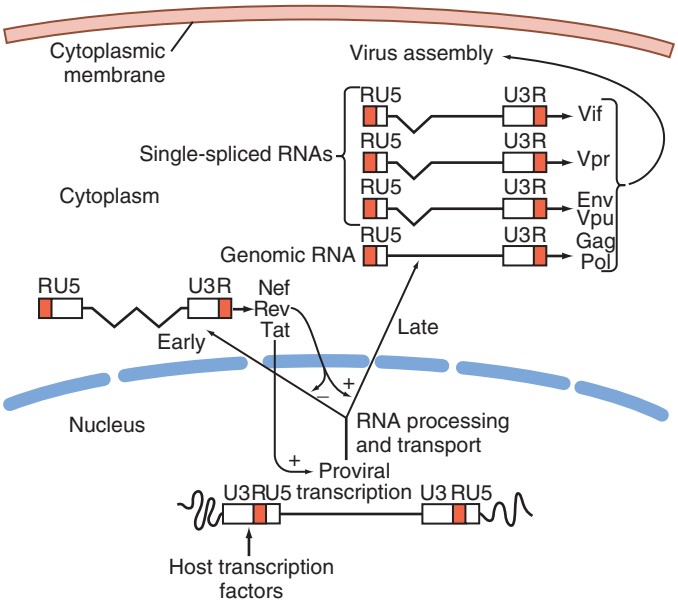

FIG. 169.5 The role of RNA splicing in the life cycle of human immunodeficiency virus type 1 (HIV-1). The early messenger RNA (mRNA) transcripts of HIV-1 are doubly spliced and produce viral regulatory proteins Tat, Rev, and Nef. The function of HIV Rev is to facilitate the expression of the late transcripts of HIV-1. These can be divided into two categories, unspliced and singly spliced. The unspliced HIV-1 mRNA has two functions: It is translated into the structural precursor polyproteins for the *gag* and *pol* gene products, and it is incorporated into virions as genomic RNA. The different single-spliced mRNAs of HIV-1 are translated into the envelope proteins gp120 and gp41, as well as Vif, Vpr, and Vpu. *Env,* Envelope; *Gag,* group-specific antigen; *Nef,* negative regulatory factor; *Pol,* polymerase; *Rev,* regulator of viral expression; *Rex,* regulator of viral expression; *Tat,* trans-activator; *Vif,* virion infectivity factor; *Vpr,* viral protein R; *Vpu,* viral protein U.

gag-pol-env genes flanked by two complete viral LTRs (see Fig. 169.2). The provirus is symmetrically flanked at either end by the viral LTR and by cellular sequences derived from the site of integration. These LTRs contain transcriptional regulatory sequences, RNA processing signals, packaging sites, and the integration sites. The 5′ end begins with the *gag* gene, which encodes core and matrix proteins; the *pol* gene, which begins in a partially overlapping reading frame encoding viral protease, RT, and IN; and then the *env* gene, which encodes the outer and transmembrane envelope proteins. A complex series of open reading frames encode the accessory proteins. The *vif* gene is contained partly within the *pol* coding region and partly downstream, and *vpr* is located downstream from *vif.* The first coding exons for *tat* and *rev* are colinear and located between *vpr* and *env*, and their second exons, located in the *env* gene, are joined to the first exons by RNA splicing. The *vpu* gene is colinear with the 5′ region of the *env* gene; *nef* is located downstream from the *env* gene and extends into the downstream LTR sequence.

Transcription of a single unspliced RNA is initiated at the 5′ end by the cellular RNA polymerase II. The unspliced mRNA serves as a template for translation of the Gag and Gag-Pol precursor polypeptides. This message is also spliced to produce single-spliced transcripts for Vif, Vpr, and Vpu proteins and the Env precursor polypeptide, and double-spliced transcripts for Tat, Rev, and Nef. The structures of these mRNAs are shown schematically in Fig. 169.5. The precursor polyproteins are then cleaved by cellular or viral enzymes; the Gag and Gag-Pol precursors are cleaved by the viral protease, which is itself transcribed from the unspliced viral message. The Env precursor polypeptide is cleaved by cellular proteases.

VIRION STRUCTURAL PROTEINS

Gag Proteins

The cleavage of the Gag precursor protein by viral protease produces the structural components of the viral core and matrix. To form the virus capsid structure, a large polyprotein is translated from the viral mRNA. This 55-kilodalton (kDa) Gag precursor protein (sometimes called p55) is cleaved into at least five structural proteins by the viral 34-kb protease encoded at the 5′ end of the *pol* gene. Lack of protease function, either through inhibition by antiretroviral drugs or after

transfection of the *p55* gene into a cell that lacks protease, results in the formation of noninfectious viral particles.[66]

The p55 protein is seen on Western blot preparations made from whole-cell lysates but not on those made from mature virions. During or shortly after self-assembly, the viral protease is activated, and the precursor is cleaved into three principal proteins and two smaller peptides. These proteins undergo extensive posttranslational modification by cellular enzymes. After translation, the initiating methionine residue is removed, p17 is myristoylated, p17 and p24 are phosphorylated by cellular kinases, and p7 (the nucleocapsid protein, or NC) binds to two zinc ions to form the zinc fingers that bind to RNA. Gag proteins are sufficient to self-assemble into noninfectious particles when expressed from transfected cells. The p17 matrix protein, MA (molecular weight, 17 kDa), contains about 130 amino acids and is myristoylated on a glycine at its amino terminus by the host cell enzyme *N*-myristoyl transferase.[67,68] The first 31 amino acids target the myristoylated p17 to the cell membrane, and virions with nonmyristoylated p17 are not infectious. In addition to viral assembly at the cell surface, MA functions as part of the preintegration complex targeting viral DNA to the cell nucleus and enabling HIV-1 to infect and integrate into nondividing cells, such as macrophages.[69] p17 has extracellular functions via binding to the IL-8 receptors CXCR-1 and CXCR-2 expressed on peripheral blood mononuclear cells (PBMCs),[70] increasing the production of proinflammatory cytokines and counteracting the inhibitory activity of IL-4 on these cells.[71]

The p24 capsid protein, CA, is produced by two cleavages to form a 240–amino-acid hydrophobic protein that forms the major subunit of the viral capsid and self-associates to form dimers and higher-order structures. This protein binds the cellular cyclophilins, a process that may be important for viral replication.[72] A major homology region of 29 amino acids is shared with many retroviruses.[73,74] p24 Gag is typically the easiest protein to detect using sera from infected patients, and serologic detection in retrovirus-infected animals gave the general name *group-specific antigen* (Gag) to these proteins. The carboxyl-terminal sequences encode a 70–amino-acid hydrophilic protein, NC, that binds both viral RNA and the capsid p24 protein, intertwining approximately one molecule with four to six nucleotides of RNA[75] and another small protein, p6. The p7 zinc-finger domain binds RNA and recognizes the packaging site on Gag.[76,77] The p6 protein appears to mediate the incorporation of Vpr into the virion and facilitates release of the virion from the cell surface, perhaps because of its binding to tumor susceptibility gene 101 (Tsg101) and ALIX (ALG2-interacting protein X), two cellular budding factors.[78] Two small peptides of unknown function, p2 and p1, are also found in the viral core.

An intracellular antiviral mechanism targets the capsid protein and is mediated by the tripartite motif (TRIM) family.[79] The TRIM motif consists of a RING (really interesting new gene) domain at the N-terminus, a B box-2 domain, and a "coiled coil" domain[80] and apparently represents a widespread and ancient innate host defense mechanism. TRIM5α appears to be functionally similar to the Friend virus susceptibility-1 (Fv1) restriction element, which regulates permissivity of murine cells of different genetic backgrounds for infection with different strains of tropic murine leukemia viruses and was identified more than 40 years ago.[81] HIV-1, however, is only partially sensitive to human TRIM5α (depending on allelic variants),[82] perhaps helping to account for its successful cross-species transmission from chimpanzees, although macaque TRIM5α strongly restricts HIV-1.[79,83] TRIM5α binds to viral capsid hexamers[84] and inhibits replication at a postentry step. The precise mechanism is not clear and may be mediated at more than one level, but may dysregulate viral capsid uncoating.[84,85]

Viral Enzymes

pol Gene Products

The *pol* gene encodes three enzymes, protease, RT, and IN. These proteins are synthesized from the same mRNA as the Gag proteins through a ribosomal translational frameshift. The cleavage of the 160-kDa precursor polyprotein is essential for viability. Because of the inefficiency of the frameshift, which is important in regulating the relative levels of viral proteins, there are about 2000 copies of each Gag protein and only 100 copies of each Pol protein per virion.

Protease

Protease is a 10-kDa 99–amino-acid protein that is fully active as a dimer. It is autocatalytically cleaved from the precursor protein during the viral assembly process. Site-specific mutagenesis has demonstrated that noninfectious particles containing uncleaved Gag and Gag-Pol proteins are produced if this enzyme is inactivated. The similarity of viral protease to other aspartyl proteases, such as angiotensin-converting enzyme, has greatly facilitated the design of potent antiviral drugs, including inhibitors of dimerization and molecules that bind to the active catalytic site.[66,86]

Reverse Transcriptase

Viral RT is an RNA-dependent DNA polymerase. This highly versatile enzyme synthesizes DNA copies from both RNA and DNA templates and degrades viral RNA from RNA-DNA hybrids. RT and its RNase H activity are required for viral replication. The protein is first cleaved from the precursor polyprotein to form a p66 homodimer and, after a second cleavage, forms a p66-p51 heterodimer with identical amino-terminal ends.

The structure of the RT heterodimer revealed the enzymatic mechanism of reverse transcription and the molecular basis of resistance to antiviral drugs.[87] The p66 and p51 assemble in an unusual head-to-tail heterodimer. Four domains of the p66 protein are similar in shape to a clenched right hand and therefore are designated as the fingers, palm, and thumb. These are joined to the RNase H domain. The cleft between them contains the highly conserved catalytic site Tyr-Met-Asp-Asp. Although the p51 subunit is derived from the same protein, it maintains a different conformation.[88,89]

RT plays a major role in the generation of genetic diversity in retroviruses. The fidelity of the enzyme has been determined for a variety of retroviruses by measuring misincorporation rates on defined templates. For HIV-1, this rate ranges from 1:1700 to 1:4000 misincorporations per nucleotide per replication, somewhat higher than for other retroviruses, and considerably greater than for host cell polymerases. For the 9.7-kb HIV-1 genome, the in vivo error rate is estimated to be one misincorporation per replication cycle.[90] Variants produced by RT generate sequence diversity that may resist immune responses or antiviral drugs or allow the virus to change cell tropism. However, the same lack of fidelity also allows nucleoside analogues to be preferentially incorporated into viral rather than cellular DNA, providing the basis for the effectiveness of RT inhibitors.

Integrase

IN is a 288–amino-acid, 32-kDa viral enzyme that mediates the linkage of double-stranded viral DNA into the host cell genome. Integration occurs after the translocation of a large complex derived from the viral core from the cytoplasm into the nucleus.[91] IN is part of this complex and catalyzes the cleavage of viral DNA and ligation to host cell DNA. A large central acidic domain of IN is highly conserved in retroviruses and retrotransposons. Once integrated, the provirus can be considered for most purposes to be a stable genetic element remaining for the life of the cell and, through cellular replication, for the life of the individual. Integrase is required for viral replication, and integrase inhibitors, such as raltegravir, are a relatively recent class of antiretroviral drugs entering clinical use (see Chapter 128).

Envelope Glycoproteins and Viral Fusion

The *env* gene of HIV-1 encodes a single-spliced viral RNA transcript that encodes both Vpu and a 160-kDa Env precursor that is synthesized in the late stages of viral replication. This 850–amino-acid Env polyprotein is cleaved by cellular proteases between amino acids 512 and 513 to form the external surface gp120 and transmembrane gp41 proteins. Proteolytic cleavage is essential for viral infectivity. There is extensive *N*-linked glycosylation on asparagine residues with high-mannose complex oligosaccharide groups. Selective removal of glycosylation sites reduces infectivity. These proteins, especially gp120, are the targets of neutralizing antibodies. The extensive variation among different strains of HIV-1, especially in gp120, and the ability of the virus to evolve during the course of a single infection and to adapt to drugs and immunologic attack rapidly present problems in therapy and vaccine

development. Most of the variability among strains of HIV occurs in the envelope sequence in five variable domains of gp120, designated V1 through V5 (comprising amino acids 128–152, 182–195, 300–330, 395–415, and 460–467, respectively).[92] The third variable region, called the V3 loop (formed by joining two cysteine residues), is a dominant antibody-neutralizing domain of gp120 and plays an important role in determining viral tropism. Four regions that are relatively invariant have been designated C1 through C4 (amino acids 33–60, 87–126, 231–276, and 460–467). These regions presumably maintain essential viral structures.

Although some structure-function relationships have been deduced from secondary structure, biochemical, mutagenic, and immunologic analyses, the solution of the crystal structure of the gp120 protein and of part of the gp41 protein has literally put a new face on the virus, with implications for cell fusion mechanisms and immune evasion. Significant progress has been made in elucidation of the structure of the intact gp120 trimer[93] by crystallization[94,95] and cryoelectron microscopy,[96] which shows a cavity-laden gp120-CD4 interface and a conserved binding site for the chemokine receptor, with evidence for a conformational change upon CD4 binding. The V3 loop, together with conserved regions that remain unexposed until CD4 binding occurs, is a principal determinant of chemokine receptor variability. An understanding of the structural basis that enables HIV to evade humoral responses, while maintaining function, may help in vaccine design.[97,98] One of the most daunting issues in designing vaccines to elicit neutralizing antibodies is the extreme variability of the outer surface of the envelope. Binding to CD4, however, exposes the coreceptor binding site, which is structurally constrained by its functional requirements. Efforts to target this region have resulted in the generation of remarkably broadly neutralizing antibodies, suggesting that this approach has promise.[99] A more recent vaccine trial[100] showed moderate efficacy that appeared to correlate with antibodies against a conserved structure in V2. Antiviral drugs, such as maraviroc, have been developed that target the gp120-CCR5 interaction.

Virus-Cell Fusion

The viral envelope is fundamentally a fusion machine that allows viral entry into target cells. Fusion depends on sequential binding of gp120 to CD4 and the chemokine receptors, but the fusogenic domain is located in gp41. The fusion peptide that is inserted into the target cell membrane is formed at the new amino terminus created by proteolytic cleavage of the gp160 precursor protein.[101] However, this hydrophobic tip must be kept in an inactive state until juxtaposed to the target cell membrane. Premature triggering of the fusion peptide would result in an inactive virus. The core structure of gp41 has been crystallized from peptide fragments.[102]

The core structure that mediates the fusion-active state between virus and cell is formed as a trimer of gp41 molecules composed of two α-helical regions within each gp41 that form a six-helix bundle characteristic of coiled coils. The crystallized complex shows striking structural homology with the low pH-induced fusogenic conformation of the influenza virus hemagglutinin protein (HA), which contains three antiparallel helices packed in a central trimeric coiled coil. The conformational change in HIV-1 is not mediated by endocytic uptake into the low pH compartment as it is for HA, however. The binding of gp120 to the second receptor likely triggers the conformational change that leads to cell fusion and virus uptake. The transition from a loop structure to a coiled coil state is the basis for the spring-loaded model of activation for membrane fusion. The fusion-active state has been identified using synthetic viral peptides to block fusion after triggering (Fig. 169.6).[103] Synthetic peptides that span all or part of these domains can inhibit HIV cell fusion and infection.[104]

Viral Regulatory and Accessory Genes

In addition to the structural genes, HIV has six accessory genes: *tat* (coding for the trans-activator of transcription), *rev* (encoding the regulator of viral expression), *vif* (encoding the virion infectivity factor), *vpr* (encoding viral protein R), *vpu* (encoding viral protein U), and *nef* (encoding the "negative" regulatory factor, a misnomer). HIV-2 and some SIV strains lack *vpu* but have *vpx*, which encodes a unique protein

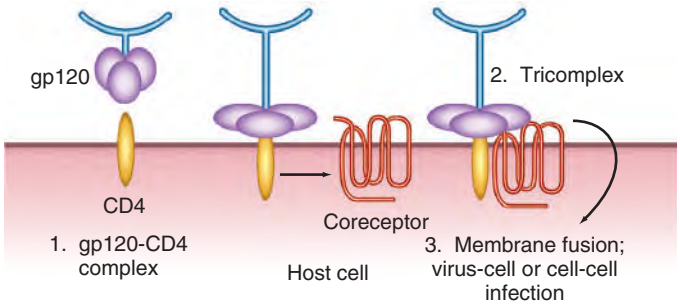

FIG. 169.6 The human immunodeficiency virus type 1 (HIV-1) infection process at the cell surface. On first infection and after initial binding of gp120 of HIV to the cell surface CD4 molecule, an envelope conformational change occurs that fosters binding to the chemokine coreceptor (e.g., CCR5 or CXCR4). This interaction involves specific portions of gp120, including the V3 region.

Vpx (viral protein X). Vpx, as mentioned earlier, targets the antiviral protein SamHD1. These genes enable the virus to usurp host cell processes and to achieve efficient replication under host-selective pressures, thus contributing to disease progression.[48] Expression of Nef in infected macrophages activates T cells through the CD2 stimulatory pathway, rendering the T cells susceptible to infection.[105] Of these genes, only *tat* and *rev* are necessary for high levels of viral expression in culture. Tat augments viral RNA levels by greatly increasing transcription, primarily by permitting elongation of otherwise blocked short nascent RNA chains. Rev regulates the splicing and transport of RNA. Vif is required for efficient cell-free transmission of virus and acts as an antagonist against APOBEC3G (apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G),[106] a deoxycytidine deaminase that acts on newly synthesized viral DNA to induce its degradation and hypermutation and that may represent a general antiretroviral defense mechanism.[107] APOBEC3G may also inhibit reverse transcription. Nef has been found to be crucial to virulence and immune evasion and downregulates surface expression of CD4, presumably to allow efficient expression of gp120 on the cell surface. As noted, expression of Nef in macrophages may be important in rendering neighboring T cells permissive to infection. Vpr also downregulates surface expression of CD4; it is the only accessory protein found abundantly in the mature virion.

Early in infection a *rev*-independent pathway removes introns from the viral transcript. The multiply spliced mRNAs for *tat* and *rev* are transported and translated and, as proteins, are shuttled back to the nucleus. As Tat acts and Rev accumulates in the nucleus, unspliced and single-spliced mRNAs for *gag, gag-pol*, and *env* and viral accessory genes *vpu, vpr*, and *vif* are transported out of the nucleus[108] (see Fig. 169.5).

The Tat protein is translated from a transcript that contains three exons, the first of which is noncoding. The *tat* reading frame overlaps both the *rev* and *env* genes. Tat is a small nuclear (14- to 16-kDa) protein that varies in size, containing 86 to 102 amino acids, depending on the strain of HIV-1. The first 72 amino acids contained in the second exon are required for full activity, whereas all the length variation occurs at the carboxyl-terminal end of the protein.[109] Tat binds to a Tat activation response element (TAR) region found in all HIV-1 mRNAs, in conjunction with cellular factors, to stabilize the nascent mRNA and enhance its rate of elongation up to 1000-fold. However, Tat activation is downregulated after periods of several hours, suggesting that these cellular cofactors may be present in limited amounts and that negative regulatory factors may downmodulate Tat function.

The Tat protein has three functional domains. A highly conserved cysteine-rich domain between amino acids 22 and 37 contains seven cysteine residues and two zinc-finger motifs, as is characteristic of a number of DNA-binding proteins. Loss of any one of six cysteines results in loss of activity. The interaction of Tat with TAR is mediated through a basic domain between nucleic acids 48 and 56. One requirement for Tat activity is the CDC2-like kinase CD9, which phosphorylates the carboxyl-terminal domain of RNA polymerase II. However, Tat must first interact with a host cell factor, which has been identified as a novel 87-kDa cyclin C–related protein named cyclin T.[110] Cyclin T is

cell specific and limits Tat activation in nonhuman cells. Tat also contains an arginine-glycine-aspartate (RGD) motif at amino acids 78 to 80 that is common to proteins that bind to integrin receptors. This feature is consistent with the finding that Tat may be secreted by cells into the medium and induces extracellular effects. Binding of Tat to cells derived from KS lesions, mediated in part through the RGD motif, enhances the growth of these cells. Tat is released from infected cells,[111] and extracellular Tat has been shown to induce KS-like lesions in nude mice.[112] Tat contains a stretch of basic amino acids that functions as a protein transduction domain, conferring the ability to be rapidly taken up into uninfected cells from the medium.[111,113] Fusion of this domain with other proteins confers the ability of those proteins to internalize into cells.[114]

All three functional domains of Tat are highly conserved among HIV-1 isolates. HIV-2/SIV Tat has an additional 30 amino acids in the amino-terminal sequence but lacks the RGD region. Tat is theoretically an inviting target for blocking viral replication, although in some situations, cellular factors may allow virus to be expressed without Tat.[115] Tat can affect the expression of heterologous and cellular genes, including the promoters of the human polyomaviruses, human papillomaviruses, and cytokines, such as tumor necrosis factor-α (TNF-α) and IL-2. The first exon of Tat downregulates class I major histocompatibility complex (MHC) expression. Another viral protein, Nef, also downregulates this protein, illustrating that single viral proteins can have multiple or complex functions that contribute to immunosuppression.

The *rev* gene encodes a serine-phosphorylated protein, Rev, that ranges in size from 106 to 123 amino acids but is most commonly 116 amino acids, with a molecular weight of 19 kDa. This protein is translated from a unique double-spliced mRNA, with the second splice acceptor located downstream from the Tat translation initiation site.[116] Expression of Rev results in the accumulation of viral structural proteins encoded by the Gag, Pol, Env, Vpu, Vpr, and Vif mRNAs. In contrast, multiple-spliced viral messages for Tat, Rev, and Nef are efficiently expressed without Rev protein. In the absence of Rev most viral mRNA is processed to multiple-spliced forms, thereby limiting the production of virions. Rev protein accumulates in the nucleolus and shuttles back and forth to the cytoplasm. Although it binds to unspliced genomic RNA, it does not appear in the mature virion. Rev binds to a unique RNA element located in the Env coding region of HIV-1 RNA. This RRE is found in all unspliced and single-spliced mRNAs.

Rev contains at least two functional domains: an arginine-rich region at amino acids 35 to 50, which is conserved and required for nucleolar localization and specific RRE binding, and a multimerization domain. After initial RRE binding, multiple additional Rev molecules bind to each other, and this oligomerization is required for activity.[117]

As a presumed example of convergent evolution, *tax* and *rex* genes present in HTLV-I (and related viruses) encode two proteins, Tax (trans-activator of transcription) and Rex (regulator of viral expression); these genes function analogously to *tat* and *rev* in HIV-1 and HIV-2. Accessory genes formed by complex splicing arrangements are not generally found in all retroviruses, suggesting that retroviruses can be classified as simple or complex, depending on the presence or absence of these genes.

The *nef* gene product is a 206–amino-acid myristoylated protein that inserts into the cell membrane. The Nef protein may have many different properties that depend in part on the experimental methodology used to analyze them. The original observation that T-cell–tropic, Nef-deleted viruses replicated to high levels led to the term *negative factor.* Downregulation of CD4 requires myristoylation, and membrane targeting of Nef to the cytoplasmic domain of CD4 increases CD4 endocytosis. The protection of HIV-1–infected cells by Nef against killing by CTL correlates with downregulation of class I MHC.[118] Nef appears to be important in maintaining high virus loads associated with rapid progression to immunodeficiency. As mentioned earlier, Nef leads to activation of T cells when it is expressed in macrophages.

The immune response to Nef may exert considerable selection pressure. When T cells capable of CTL activity against Nef were transferred to HIV-infected patients, a Nef-deleted variant emerged and, although there was apparent successful immunologic intervention, the patient's disease progressed.[119] Nef-deleted viruses detected in several human clusters were originally associated with little or no disease, raising the possibility that they represented less virulent viruses.[120] However, long-term follow-up of these cases has demonstrated that definite, albeit slower, disease progression does indeed occur.[121]

Vif is a 193–amino-acid viral protein of 23 to 27 kDa with no *N*-linked glycosylation sites. The infectivity of Vif deletion mutants is decreased up to 1000-fold in some cell lines compared with that of wild-type virus. The deleted virus is capable of cell entry and initiating reverse transcription, but dsDNA is not produced. As noted, Vif blocks the antiviral activity of deoxycytidine deaminase APOBEC3G.[106]

Vpr is a 96–amino-acid protein translated from a single-spliced mRNA and, like Vif, its expression is dependent on Rev function. Vpr is abundantly present in the mature virion associated with capsid protein. Expression of p55 Gag and Vpr in transfected cells is sufficient for incorporation and export of viral proteins. Vpr plays a role in the nuclear localization of the preintegration complex.[122,123] In addition, in transfected human muscle cells, Vpr blocks proliferation and induces differentiation, suggesting a nuclear role for Vpr in regulation of gene expression. Vpr causes arrest of cell-cycle progression at the G_2/M interface, presumably through an effect on cyclin CDC2 activity, which correlates with the ability to activate HIV transcription.[124,125] In addition, Vpr causes massive ruptures in cell nuclei.[126]

Vpu is an amphipathic integral membrane protein.[127] The first 27 amino acids are hydrophobic, whereas the remainder of this small, 81–amino-acid 16-kDa protein is hydrophilic. A single-spliced message overlaps with the *env* gene in a different reading frame.[128] The protein forms oligomeric complexes localized to the perinuclear region. Cells infected with Vpu-defective mutants show large accumulations of intracellular vesicles, in contrast to those infected with wild-type virus. Vpu, along with Nef, is associated with the rapid degradation of CD4, which may in part eliminate CD4-gp160 intracellular complexes that interfere with virus production. The CD4 cytoplasmic tail is required for targeting the degradation of CD4 by Vpu. Vpu has structural similarities to the influenza virus M2 protein, an ion channel protein that modulates the pH of the trans-Golgi.[129] Perhaps more important, Vpu causes the ubiquitination and degradation of tetherin, an IFN-dependent antiviral protein that impedes viral release from the cell surface.[130]

Virus Regulation and the Long Terminal Repeat

The LTR of all retroviruses, located at each end of the provirus as a direct repeat containing the U3, R, and U5 regions, functions as an eukaryotic transcription unit. The U3 region contains the viral promoter and enhancer elements. The R region includes the mRNA initiation site (+1) and ends at a polyadenylation termination site. The function of the U5 region is not well understood. It separates the R region from the tRNA primer binding site used to initiate reverse transcription. HIV-1 uses $tRNA^{lys}$ as a primer. Once the virus has formed an integrated DNA copy, it depends on cellular machinery for transcription and translation. However, the control of virus expression results from a complex set of interactions between viral elements and cellular proteins. Small changes in these regions may result in profound differences in virus behavior. Cis-acting control elements of the virus (TAR, TATAA) and enhancer and negative regulatory regions (located within the U3 and R regions) interact with cellular and viral proteins. These interactions, which occur at both the DNA and the RNA levels, are crucial in controlling the level of viral expression in both resting and activated cells.

The TATAA box, located at –27 (relative to the RNA initiation site), binds the critical cellular transcription factor IID (TFIID) to initiate transcription. The promoter region, the binding site of the cellular polymerase, lies further upstream 5′ (between –45 and –77) and contains three binding sites for the cellular specificity protein 1 (SP1) transcription factor.[131] An enhancer element is still further upstream 5′, mapping to nucleotides –82 to –105, and contains a consensus sequence also found in the immunoglobulin κ, IL-2, and IL-2R enhancer regions. This region binds an inducible cellular transcription factor, NF-κB. Although originally described in B lymphocytes, this factor or family of factors is also expressed in activated T cells and stimulates HIV expression.[59,132] In addition to NF-κB, other factors can increase HIV-1 promoter activity by interactions in the region. These include cellular cytokines, such as

TNF-α and IL-1, and heterologous viral proteins, such as HTLV-I Tax. Such observations indicate molecular mechanisms whereby other viruses could interact with HIV; however, the relevance of such interactions in vivo is unknown.

Further upstream 5′ are the binding sites for additional cellular factors (activating protein-1 [AP-1], nuclear factor for activated T cells-1 [NFAT-1]) that lie within a negative regulatory element (NRE). Removing the NRE from a functional provirus enhances virus expression, again suggesting that viral production is carefully modulated both negatively and positively.

The R region of the LTR codes for the 5′ untranslated leader sequence shared by all HIV-1 mRNAs and for the TAR, an essential binding site for the potent virally coded HIV-1 trans-activating protein, Tat. The TAR in the LTR transcript forms a unique stem-loop structure. Of interest, the structure of the HIV-2 LTR is significantly different from that of the HIV-1 LTR and may contribute to the distinctly different biologic activities of these two human retroviruses.

VIRUS-HOST INTERACTIONS

Viral Receptors, Chemokines, Receptors, and Tropism

Chemokines and their receptors constitute a complex signaling system essential for orchestrating angiogenic, inflammatory, and chemotactic responses. More than 40 chemokines are grouped into two principal families, C-C and C-X-C; there are at least 14 known seven-transmembrane–spanning G protein–coupled chemokine receptors. These molecules have been exploited by some bacterial and viral pathogens as their receptors to gain entry to or activate cells, and virally encoded antagonists often subvert chemokine function.[133,134]

The revelation that chemokine receptors are essential for HIV cell fusion has brought together several distinct areas of viral research: how $CD8^+$ T cell–derived factors suppress HIV-1 replication, the mechanisms of cell entry and viral tropism, and host genetic determinants of infection. Three CC (or β) cytokines released by $CD8^+$ T cells—RANTES, MIP-1α, and MIP-1β—bind to the CCR5 receptor and potently suppress HIV macrophage-tropic virus.[135,136] The first coreceptor identified, the CXCR4 molecule, known at the time only as an "orphan receptor," was discovered using a complementary DNA screening approach for receptor activity mediating cell-cell fusion.[39] A group of reports rapidly followed, showing that a recently identified receptor, CCR5, which could use the identified suppressor molecules as ligands, was the main coreceptor for primary isolates and macrophage-tropic strains.[40–43] Dual-tropic strains can use both coreceptors.[137] Chemokine coreceptor use may be a determinant of viral virulence and disease progression.[138] In addition, studies have pointed to the critical role of cholesterol in the cell membrane in HIV-1 coreceptor function; removal of cellular cholesterol rendered primary cells and cell lines highly resistant to HIV-1–mediated syncytium formation and to infection by CXCR4- and CCR5-specific viruses.[139]

A mutant CCR5 gene *(CCR5delta32)* that encodes a defective protein and is unable to bind virus has been found in exposed but uninfected persons, strongly suggesting that a functional CCR5 protein is required for infection.[140] Homozygosity for this mutant[141] is a strong protective factor, although rare infections by CCR5-independent viruses have been documented in a person with hemophilia and in another person after sexual transmission. Of interest, the lack of CCR5 does not have apparent major negative health consequences. Heterozygous adults and children are not protected from infection but may take a longer time to develop disease.[142,143] An interesting example of the potential for CCR5-negative cells to resist infection is the so-called "Berlin patient,"[144] who was HIV infected and developed leukemia as well. Myeloablation was performed, followed by transplantation with hematopoietic stem cells from an individual with a homozygous *CCR5delta32* genotype. He has remained free of virus, perhaps due to a combination of nonpermissivity of donor cells and graft-versus-host killing of residual infected host cells. This suggests a potential mechanism for achieving a "viral cure" (see later).

Pathogenesis, T-Cell Depletion, and Viral Load

Understanding the rates of HIV-1 production and associated loss of T cells has been dramatically advanced by the ability to measure changes produced by potent new drug combinations. HIV-1 production and T-cell turnover constitute a continuous dynamic process.[145,146] Mathematical modeling of virus production has suggested that a continuous battle is being waged between the virus and host. Production of billions of virions and T-cell turnover, estimated at 1 billion cells/day, may help account for the very rapid emergence of viral variants and the fluctuating and progressive nature of T-cell depletion. Virus may be distributed across different cellular compartments, each with different rates of turnover and production. Even in the steady state that may occur during periods of clinical latency, large numbers of virus freshly infecting T cells lead to a highly activated immune system that is attempting both to control virus replication and to renew itself.[147] The increase in $CD4^+$ T cells with antiretroviral therapy (ART) is probably due to the result of a combination of initial redistribution of memory T cells and a continuous but slow repopulation with newly produced naïve T cells.[148] The mathematical models that have been proposed, however, grossly oversimplify the real dynamics of infection and are no longer widely accepted as a valid major explanation for HIV pathogenesis and T-cell loss. Several other pathologic mechanisms, including indirect viral killing and activation-induced apoptosis, have been suggested to play a role. Uninfected $CD8^+$ T cells turn over as rapidly as $CD4^+$ T cells but are not initially depleted.[149] The eventual loss of noninfected $CD8^+$ T cells may be mediated by gp120 binding to CXCR4.[150] Eventually, viral escape from immune control and emergence of T-cell–tropic viruses using CXCR4 may lead to immunodeficiency,[151] although progression to AIDS often occurs in the absence of detectable variants using CXCR4. All these models incorporate two common assumptions—a demonstrated quantitative association between virus production and T-cell depletion, and a compartmentalization of virus production and cell turnover at different rates.

The significance of virus integration for the natural history of HIV-1 infection has been vividly demonstrated by the effects of combination therapy on virus production from different populations of cells and has helped define what is meant by latent infection. Potent antiretroviral regimens that include a combination of RT and protease inhibitors can produce sustained reductions of plasma viral RNA to below detectable limits.[152] Treated patients with detectable viral plasma RNA, even if the level is greatly reduced, have viral loads in their lymph nodes similar to those in the nodes of patients who do not receive treatment, reflecting ongoing viral replication and emergence of drug resistance.[153] However, even in those with no detectable plasma RNA, viral DNA could still be detected in lymph nodes and PBMCs, and virus could be grown from PBMCs after removal of $CD8^+$ cells and activation. Furthermore, no new mutations associated with drug resistance were detected from these isolates recovered after 2 years of therapy. This strongly suggests that virus persists in a long-lived and latently infected T-cell population. This pool of latently infected cells is probably established very early during primary HIV-1 infection. Even though plasma viremia could be suppressed, initiation of ART as early as 10 days after onset of symptoms did not prevent generation of latently infected $CD4^+$ lymphocytes.[154] Although the frequency of these cells is low, on the order of 16/million PBMCs, the fact that hidden virus may survive in these cells may represent a significant factor in long-term therapy and may shape strategies to eliminate virus.[155] Indeed, therapy may need to be continued for 60 to 70 years to eradicate the reservoir of long-lived latently infected cells. One alternative has received consideration with the advent of convenient gene editing tools, such as clustered regularly interspaced short palindromic repeats (CRISPR), along with the example of the Berlin patient. In principle, ex vivo editing to cripple both copies of the *CCR5* gene from hematopoietic stem cells, followed by transplantation into patients, could result in a virologic cure. Some success in this approach has been reported in humanized mouse models of HIV infection,[156] but there are obviously formidable technical hurdles, such as off-target effects, efficiency, and expense to be overcome.

Similar to the impairment or killing of infected cells, there are several distinct mechanisms whereby bystander cells can be affected in vitro. For example, HIV-1–infected PBMCs taken directly from patients or obtained through infection of normal PBMCs with HIV-1 in vitro have shown a marked impairment of proliferative responses, even though only a small fraction of T cells are infected.[157–159] Other possible indirect

(bystander) effects include impaired hematopoiesis, leading to cytopenias of several blood cell lines, impaired thymopoiesis, and anergy; apoptosis of uninfected immune cells, likely through increased levels of cytokines, such as TNF-α, IFN-α, and transforming growth factor-β; and hyperactivation and apoptosis of uninfected immune cells by the effects of extracellular Tat.

Viral Variation: Genetic and Phenotypic Variation

On the basis of phylogenetic analysis, HIV strains have been separated into major (M) subgroups and more distant groups N and O. Within the M group are multiple subtypes A, B, C, D, F, G, H, J, and K and ever more numerous circulating recombinant forms, including CRF 01 AE/B and CRF 02 A/G.[160] A subspecies of chimpanzees *(Pan troglodytes troglodytes)* has been identified as the likely source of HIV-1.[161,162] Zoonotic transmission appears to have occurred on at least three occasions, with one such transmission in southeastern Cameroon being the origin of the most widespread HIV-1 group, HIV-1(M). It is likely that this has happened on other occasions, but that the transmitted viruses failed to survive as infectious human agents, and the three reported cross-species transmissions are the only ones that have been successful. Data has been recently presented[163] suggesting that after its introduction into humans in Africa, HIV-1 was introduced into the United States in the 1960s, from where it spread to many other countries, giving rise to clade B HIV-1(M), which is highly prevalent in the United States, Europe, and other areas. Clade C is the most prevalent in many parts of Africa and in India.

One of the most striking characteristics of HIV-1 is its remarkable variability, contributing to phenotype diversity and resulting in altered cell tropism, immune escape, and resistance to RT and protease inhibitors. As a consequence of the underlying variation by mutation, high rates of virus turnover and selection of viral variants cause viral evolution in individual hosts with time, as well as among populations of infected individuals. Most affected persons were thought to be infected with a single strain that evolves into a swarm of related viruses or quasispecies during the course of infection.[164] Recently, however, deep sequencing and phylogenetic analyses of viruses from recently infected individuals have suggested that only about two-thirds of individuals are infected with a single virus, with the remainder being infected with from three to five genetically distinct viruses, called transmitted/founder viruses.[165]

Most of the variation is neutral and not adaptive. Although the mutation rate per base pair per cycle is presumed to be equal throughout the genome, diversity is greatest within distinct regions of the envelope gp120, presumably because of selection and relatively low fitness costs. HIV is going through rapid epidemiologic changes, even as the virus is being studied. Thus genetic relatedness of viruses can be used to track transmission of the virus as well as the relatedness of different viruses. This information is often presented in the form of viral phylogenetic trees.[166]

MALIGNANCIES AND HUMAN IMMUNODEFICIENCY VIRUS

Although cancer is not common in the young adults most frequently exposed to HIV, KS has been a defining clinical presentation of AIDS (see Chapter 140). Previously known as a rare and usually indolent vascular tumor, the incidence of KS in patients with HIV infection early in the epidemic was 10% to 20%, more than 10,000 times that in the general population. Several forms of KS have been described: classic KS, endemic KS, non–HIV-associated KS, transplantation-associated KS, and AIDS-associated KS. The tumor appears as a vascular proliferation characterized by the presence of spindle cells, vascular channels, and a mixed cellular infiltrate. The "malignant" cell is most likely a population of activated endothelial cells, which are sometimes clonal and sometimes not. These cells themselves and factors produced from them, such as basic fibroblast growth factor and vascular endothelial cell growth factor, can induce vascular lesions in nude mice. The HIV protein Tat can bind to KS spindle cells and stimulate their growth.[167] Moreover, Tat can cooperate synergistically with a G protein–coupled receptor encoded by human herpesvirus 8 (HHV-8; see later) in inducing KS-like lesions in nude mice.[168] Although the importance of immunodeficiency and viral proliferation in the etiology of KS is well established, the mechanism(s) is not certain, and KS may occur early in the course of HIV infection.

Within the group of HIV-infected individuals, KS occurs predominantly in homosexuals and bisexuals, leading to the hypothesis that a previously undescribed infectious agent might cause KS. In 1994, using DNA subtraction hybridization, DNA representing a novel herpesvirus was obtained from KS lesions, which led to the isolation of HHV-8[169] (see Chapter 140). Also known as KS herpesvirus, it is related to other gammaherpesviruses such as Epstein-Barr virus (EBV). HHV-8 has also been identified in an EBV-transformed B-cell line and from a unique form of B-cell lymphoma known as body cavity lymphoma. This large virus contains many genes that interact with host cells, including several chemokine and cytokine homologues that can induce vascular growth.[170] Seroepidemiologic studies have made a strong association between this virus and KS. HHV-8 was found to be associated with all forms of KS, and seroconversion precedes the appearance of KS in HIV-infected persons. The prevalence of HHV-8 is far greater than the incidence of KS, however, and the relationship of the virus to the disease is not likely to be simple. HHV-8 thus appears to be necessary but not sufficient to cause KS. A review discusses this and other aspects of KS that are not clear at present.[171] The incidence of KS in AIDS in developed countries continues to decrease.

B-cell lymphoma, often associated with EBV, is the second most common cancer associated with AIDS, occurring in approximately 3% to 4% of individuals as their first AIDS-defining diagnosis. One type, primary effusion lymphoma, is caused by HHV-8. The most common lymphoma is that of the brain (primary brain lymphoma). However, in contradistinction to KS, lymphoma appears to be a late manifestation of HIV disease, with rates rising directly with length of time infected. Up to 16% of AIDS patients eventually die from this condition. In 53,042 AIDS patients from 21 European countries, approximately 2.6% of injection drug users, 3.2% of transfusion recipients, 3.4% of homosexual men, 3.9% of hemophiliacs, and 2.6% of those who acquired HIV through heterosexual contact eventually developed AIDS-related lymphoma.[172]

In the United States, data from the Multistate AIDS/Cancer Match Registry have demonstrated a significantly increased risk of lymphoma in patients with a prior AIDS diagnosis, compared with population control subjects who had not been diagnosed with AIDS.[173] The relative risk of all non-Hodgkin lymphoma (NHL) was increased approximately 113-fold. Although the greatest risk is for NHL, there is a 10-fold increase of Hodgkin disease in the infected group as well.

Because of its persistent presence in lymph nodes, even in the absence of detectable viral RNA,[174] and because it activates signal transduction in B cells,[70] the viral p17 has attracted interest as a possible contributor to lymphomagenesis. These tumors are predominantly extranodal and often of high grade, with small cell noncleaved histologic features.[175] EBV is found in almost all primary CNS tumors, but high rates of dysregulated B-cell turnover may predispose to lymphoma. Rates of EBV-associated primary brain lymphoma have fallen off markedly in the era of ART, but the same cannot be said for systemic lymphoma, which appears to be increasing in frequency as people live longer with HIV.[176]

Several other EBV-associated rare tumors, including leiomyosarcoma, have also been reported.[177] Cervical cancer, almost universally associated with human papillomavirus (HPV) infection, is accepted as an AIDS-defining illness.[177] High rates of HPV-associated anal intraepithelial neoplasia (AIN) have been documented among HIV-infected homosexual men, and routine screening for AIN is being considered, using the same methodology as Pap smears, with samples obtained through anoscopy.[178] The incidence of many common cancers does not appear to be greatly increased among HIV patients, and many of the incidence increases are among cancers associated with other viruses, although it should be noted that non-AIDS cancers are increasing as the infected population ages.

CONCLUSION

The widespread and successful use of ART directed at HIV-1 has continued to develop rapidly (see Chapter 128). Significant advances in the ability to treat individual cases, propelled by an understanding of the biology of the virus, have led to the control of viral replication and have altered the course of disease progression. At present, patients have been maintained on combination therapy for years, with a low

rate of relapse because of the development of resistance and low viral burdens. Immune restoration and long-term biologic control or complete eradication of the virus with minimal toxicity remain elusive goals, but intense efforts continue to focus on new anti-HIV therapies. As we have learned, retroviral disease has important social aspects that, as much as viral biology, determine the extent of viral transmission and the dimensions of the epidemic; this results in a series of epidemics, each with a unique sociobiology. Moreover, despite remarkable progress, social and economic effects of the global HIV epidemic have materialized vividly in sub-Saharan Africa, with devastating losses of population life expectancy and productivity. Renewed emphasis on the development of an effective anti-HIV vaccine that might stem an ongoing epidemic or ameliorate the course of disease has been the subject of intensive study in nonhuman primates and in clinical trials (see Chapter 119). The history of retrovirology has been filled with remarkable, often serendipitous, discoveries based on scientific imagination supported by technologic advances. There is every reason to expect that continued advances will deepen our understanding of fundamental biologic processes and that we will meet the challenges presented by HIV and the inevitability of the emergence of new pathogens.

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170 Introduction to the Human Enteroviruses and Parechoviruses

José R. Romero

Members of the genera *Enterovirus* and *Parechovirus* are picornaviruses. Picornaviridae is a large family of morphologically identical, single-stranded, positive-sense RNA viruses that share similar genomic and structural organizations. Originally the human enteroviruses were divided into five species on the basis of differences in host range and pathogenic potential: polioviruses, group A coxsackieviruses (CV-A), group B coxsackieviruses (CV-B), echoviruses (E), and newer enteroviruses (EVs) (Table 170.1).[1,2] All enteroviruses discovered since 1970 have been assigned to the enterovirus species.

A classification scheme based on RNA homology within the VP1 capsid protein coding region that replaces the traditional classification divides the genus *Enterovirus* into four species, designated enterovirus A through D (Table 170.2).[2,3] Isolates of the same serotype characteristically diverge in the VP1 region by less than 25% within corresponding nucleotide sequences and by less than 12% within amino-acid sequences.[3] Of the original 72 serotypes identified, 64 remain after recognition of redundant serotypes and reclassification of others (see Table 170.1).[2] Today more than 110 unique enterovirus serotypes known to infect humans have been identified, each distinguished from one another on the basis of phylogeny of the VP1 coding region.[3] Parenthetically, the rhinoviruses, once a genus unto themselves, have been reassigned as species within the genus *Enterovirus* (see Chapter 175).[2]

A more recently established genus, *Parechovirus,* includes four species (parechovirus A [formerly human parechovirus], parechovirus B [formerly Ljungan virus, a rodent picornavirus], parechovirus C, and parechovirus D).[4] Two serotypes formerly considered to be in the echovirus species, E-22 and E-23, have been reclassified as members of the parechovirus A species, human parechovirus 1 and 2 (HPeV1 and HPeV2),[4,5] on the basis of certain biophysical and biochemical properties that differ from those of enteroviruses (see later discussion). In addition, at least 19 new serotypes of HPeV have been described and are discussed in Chapter 173.

ENTEROVIRUSES

Virology

Physical Characteristics

Enterovirus virions are nonenveloped, icosahedral capsids of approximately 30 nm composed of 60 structural subunits; the subunits are formed from four polypeptides, with an aggregate molecular weight of 80 to 140 kDa, that surround the single-stranded RNA genome.[6] In contrast to other picornaviruses (e.g., rhinoviruses), the human enteroviruses are stable over a wide range of pH (3–10), permitting them to retain infectivity during transit through the gastrointestinal tract. Lacking a lipid envelope, enteroviruses are resistant to ether, chloroform, and alcohol. However, they are readily inactivated by ionizing radiation, formaldehyde, or phenol.[6] Molar magnesium chloride reduces the thermolability of enteroviruses across a wide range of temperatures; this feature allows live-attenuated oral poliomyelitis (OPV) vaccines to maintain potency when refrigeration is suboptimal or unavailable.

The capsid encloses a linear RNA genome of approximately 7.5 kb that is divided into four regions: a 5′ nontranslated region of approximately 740 nucleotides, a continuous-coding region of approximately 6625 nucleotides, a 3′ nontranslated region of approximately 70 nucleotides, and a 3′ poly(A) tail of 70 to 100 nucleotides in length.[7,8] The 5′ terminus is covalently linked to a small virus-coded protein (VPg), which is required for the initiation of RNA synthesis. The 5′ nontranslated region contains genomic elements and sequences essential for viral RNA replication, translation, and, for some enteroviruses, virulence. The most conserved region of the genome among the human enteroviruses is the 5′ nontranslated region. The regions coding for the structural proteins show the greatest intraserotypic sequence variability, in particular, within the coding regions for epitopes that bind neutralizing antibody. The 3′ nontranslated region plays a role in viral RNA replication. Removal of the poly(A) 3′ terminus renders the RNA noninfectious.

Molecular Biology

The RNA genomes of naturally occurring polioviruses, attenuated polioviruses, and many nonpolio enteroviruses have been fully sequenced, and the replication of polioviruses in primates and transgenic mice has been studied in extensive detail.[7,8] Full-length genomic clones of the polioviruses and several enteroviruses have provided the tools for the dissection of many of the molecular events of infection.[7,8,9] The molecular structure and intracellular replicative events appear to be similar for all four enteroviruses species A through D.

Host cell susceptibility to enteroviral infection is defined in large part by the presence of specific membrane receptor proteins that serve as cellular receptors or coreceptors that bind enteroviruses generally along taxonomic lines (Table 170.3).[10,11] The three poliovirus serotypes share a common receptor (poliovirus receceptor [PVR] or CD155), a member of the immunoglobulin superfamily that is coded on human chromosome 19.[12,13,14] The receptor and coreceptor proteins have been identified for many of the human enteroviruses. Both decay-accelerating factor (DAF, or CD55), a complement regulatory protein, and intercellular adhesion molecule 1 (ICAM-1) play a role in CV-A21 cell entry.[15] The CV-B serotypes also interact with two different cell membrane proteins, the 49-kDa coxsackievirus-adenovirus receptor (CAR) and DAF.[16,17] The presence of CAR permits binding and cell entry by all six CV-B serotypes,[17] whereas antibodies to DAF block binding and infection by serotypes 1, 3, and 5.[16,18,19] DAF also appears to be a major echovirus receptor, binding multiple echovirus serotypes,[11,20] whereas serotypes E-1 and E-8 bind to the α_2-subunit of very late antigen 2 (VLA2) integrin molecule.[21,22] P-selectin glycoprotein ligand 1 (PSGL-1) and the scavenger receptor class B member 2 (SCARB2) are both used as receptors for EV-A71.[23,24] The receptor for EV-D68 has been identified as ICAM-5/telencephalin.[25]

The processes of penetration, uncoating, and release of the nucleic acid into the cytoplasm occur within minutes at 37°C. Binding of poliovirus with its cellular receptor leads to conformational changes in the viral capsid that allow for extrusion of VP4 and the formation of a channel through which the RNA genome enters the cytoplasm of the cell.[8] RNA synthesis begins within 30 minutes, leading to an exponential increase of minus-strand complementary and plus-strand progeny RNA until 2.5 hours after infection, when there is a switch to a linear accumulation of mainly progeny RNA.[8] The full-length RNA functions as a monocistronic messenger whose translational product, a polyprotein of 250-kDa molecular weight, is encoded by a single open reading frame

TABLE 170.1 Conventional Classification and Host Range of Human Enteroviruses

SUBGENERA	NO. OF SEROTYPES	HOST RANGE[a]		
		Primates	Newborn Mice	Cell Culture
Polioviruses[b]	1–3	++	0	++
Group A coxsackieviruses[c,d]	1–24	0	+++	±
Group B coxsackieviruses[c,e]	1–6	0	+++	++
Echoviruses[f]	1–34	0	0	++
Enterovirus[g,h]	68–72	Variable	Variable	Variable

[a]Replicative capacity.
[b]Polioviruses generally replicate only in primates or primate cell cultures, although rare strains such as the type 2 Lansing strain have been adapted to rodents. Although poliovirus multiplies in the alimentary tract of some nonhuman primates, the hallmark of these viruses is the characteristic histopathologic lesions produced by direct inoculation of the central nervous system.
[c]The coxsackieviruses were first recovered from the feces of children with poliomyelitis in the town of Coxsackie, New York.[144] In contrast to polioviruses, they produce paralysis and death in experimentally infected suckling mice.
[d]All group A coxsackieviruses produce generalized myositis of skeletal muscle and flaccid hind limb paralysis in suckling mice,[145] and coxsackievirus A7 is pathogenic for the primate central nervous system. However, most group A coxsackieviruses except serotypes A9 and A16 grow poorly in cell culture. Coxsackievirus A23 has been reclassified as echovirus 9, leaving 23 coxsackievirus serotypes.
[e]Group B coxsackieviruses are distinguished by their ability to produce focal myositis and generalized infection of the myocardium, brown fat, pancreas, and central nervous system in suckling mice, resulting in spastic paralysis. The group B coxsackieviruses are commonly isolated in cultured primate cells.
[f]The echoviruses (enteric cytopathic human orphan viruses) were originally discovered in fecal specimens of healthy children.[146,147] They cause cytopathic effects in primate cell culture but are generally nonpathogenic for suckling mice (except for echovirus 21) and primates. Echovirus 10 has been reclassified as reovirus 1, and echovirus 28 has been reclassified as rhinovirus 1. Echovirus 34 is a variant of coxsackievirus A24. Echoviruses 22 and 23 have been assigned to the genus *Parechovirus* as parechovirus serotypes 1 and 2, respectively.[89] Therefore, 29 of the original 34 serotypes of echovirus remain.
[g]The human enteroviral serotypes recognized since 1970 are designated by serial numbers only.[148]
[h]Hepatitis A virus was briefly classified as enterovirus 72 until genetic sequence data led to its reclassification as a hepadnavirus.[149]

TABLE 170.2 Classification of Human Enteroviruses by Partial Sequencing of VP1

SPECIES	SEROTYPES
Enterovirus A	CV-A2, CV-A3, CV-A4, CV-A5, CV-A6, CV-A7, CV-A8, CV-A10, CV-A12, CV-A14, CV-A16 EV-A71, EV-A76, EV-A89, EV-A90, EV-A91, EV-A92, EV-A114, EV-A119, EV-A120, EV-A121
Enterovirus B	CV-A9, CV-B1, CV-B2, CV-B3, CV-B4, CV-B5, CV-B6 E-1, E-2, E-3, E-4, E-5, E-6, E-7, E-9, E-11, E-12, E-13, E-14, E-15, E-16, E-17, E-18, E-19, E-20, E-21, E-24, E-25, E-26, E-27, E-29, E-30, E-31, E-32, E-33 EV-B69, EV-B73, EV-B74, EV-B75, EV-B77, EV-B78, EV-B79, EV-B80, EV-B81, EV-B82, EV-B83, EV-B84, EV-B85, EV-B86, EV-B87, EV-B88, EV-B93, EV-B97, EV-B98, EV-B100, EV-B101, EV-B106, EV-B107, EV-B110, EV-B111, EV-B112, EV-B113
Enterovirus C	PV-1, PV-2, PV-3 CV-A1, CV-A11, CV-A13, CV-A17, CV-A19, CV-A20, CV-A21, CV-A22, CV-A24 EV-C95, EV-C96, EV-C99, EV-C102, EV-C104, EV-C105, EV-C109, EV-C113, EV-C116, EV-C117, EV-C118
Enterovirus D	EV-D68, EV-D70, EV-D94, EV-D111, EV-D120

CV-A, Group A coxsackievirus; *CV-B,* group B coxsackievirus; *E,* echovirus; *EV,* enterovirus; *PV,* poliovirus.
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TABLE 170.3 Enterovirus and Parechovirus Cell Membrane Receptors

	RECEPTOR PROTEIN
Enteroviruses (Serotype)	
Polioviruses 1–3	Poliovirus receptor (PVR)
Coxsackieviruses A13, A18, A21	Intercellular adhesion molecule 1 (ICAM-1)
Coxsackieviruses B1–B6	Coxsackie-adenovirus receptor (CAR)
Coxsackieviruses B1, B3, B5	Decay accelerating factor (DAF)
Echoviruses 1, 8	Very late antigen 2 ($\alpha_2\beta_1$)
Echoviruses 6, 7, 11–13, 20, 21, 29, 33	DAF
Enterovirus C70	DAF
Enterovirus A71	P-selectin glycoprotein ligand 1 (PSGL-1) and scavenger receptor class B member 2 (SCARB2)
Parechoviruses	
Human parechovirus 1	$\alpha_v\beta_1$, $\alpha_v\beta_3$, integrins

involving about 90% of the entire genome. By convention, the enteroviral polypeptide is organized into three large regions, organized 5′ to 3′ as P1, P2, and P3.[26] The nascent polypeptide is cleaved as it emerges from the host ribosome by viral proteins acting initially in *cis* and later in *trans* to yield the proteins, and protein intermediates are essential to the viral life cycle.[8,26] Cleavage of the P2 and P3 regions results in eight nonstructural proteins and several protein intermediates, whose known functions include polymerase activity, proteolytic cleavage of the translational products, inhibition of host cell protein synthesis, and cellular remodeling.[8]

Following cleavage of P1 from the nascent polypeptide, it undergoes further cleavages to form the viral capsid, which proceeds by aggregation of five copies each of VP1, VP3, and VP0 (the precursor of VP2 and VP4) into subunits and assembly of 12 of these pentamers into the complete dodecahedral capsid shell. Encapsidation of the viral RNA is associated with a final cleavage of the VP0 protein to VP2 and VP4. The latter is an internal protein closely associated with the RNA. The complete virion contains 60 copies of each of the four structural proteins.[8]

Host protein and RNA synthesis are severely compromised by 3 hours after infection. After about 6 to 7 hours, virions are visible by electron microscopy within the cytoplasm, and they are subsequently released by lysis of the cell, resulting in a yield of 10^4 to 10^5 virions per cell. The number of infectious virions is 10- to 1000-fold lower.[8]

Pathogenesis and Immunity in Enteroviral Infections

Pathogenesis

The pathogenesis of poliovirus infection has been extensively investigated in primates experimentally infected with neurovirulent strains and in humans infected with vaccine strains,[27–30] and it is widely assumed that the early pathophysiologic events of nonpolio enterovirus infections are similar. Studies of coxsackievirus infection in mice have produced much information about the influence of various host and environmental

factors on the ability of the virus to replicate in the heart, brain, and other organs and about the mechanism of vertical transmission of enteroviruses from infected pregnant animals to their offspring.

Enteroviruses infect humans via direct or indirect contact with virus shed from the gastrointestinal tract or upper respiratory tract. Whereas ingested virus implants and replicates in the pharynx and the distal small bowel, volunteer studies have shown that attenuated polioviruses replicate most efficiently in the distal small intestine.[27] The precise site of viral entry has long been the subject of conjecture. Studies have demonstrated that microfold cells (M cells) expressing the PVR serve to transport polioviruses across the intestinal mucosa.[31,32] Enteroviral replication in ileal lymphoid tissue is detectable 1 to 3 days after the ingestion of virus. The quantity of virus recoverable from the tonsils is much less than that in Peyer patches, where it may reach 10^7 to 10^8 tissue culture median infective doses ($TCID_{50}$) per 1 g. In healthy individuals, the duration of viral excretion is typically less than 3 to 4 weeks from the pharynx and 5 to 6 weeks in the feces. Longer periods are reported in individuals with B-cell immunodeficiencies and, occasionally, in healthy children.[33,34,35,36]

After multiplication in submucosal lymphatic tissues, enteroviruses pass to regional lymph nodes and give rise to a transient minor viremia. This leads to infection and viral replication in reticuloendothelial tissue including liver, spleen, and bone marrow. The most common result is a subclinical infection, in which viral replication is contained by host defense mechanisms. In a minority of infected persons, however, further replication of virus occurs in these reticuloendothelial sites, leading to a sustained major viremia that coincides with the onset of the minor illness of poliomyelitis and probably of the nonspecific febrile illnesses associated with other enterovirus infections. Prodromal viremia has been demonstrated with wild strains of poliovirus[30,37] and E-9[38] but is uncommon with Sabin OPV strains except for type 2.[39]

The major viremia results in dissemination to target organs such as the central nervous system, heart, and skin, where tissue necrosis and inflammation occur in proportion to the level of viral replication. Histopathologic lesions are usually not seen in the gastrointestinal tract, even though the small bowel is the site of initial viral replication. The severity of infection in experimental animals can be enhanced by induced exercise, cold exposure, malnutrition, pregnancy, and immune suppression with corticosteroids or radiation.

Viral Mutation During Natural Infection

Enteroviruses undergo a high rate of mutation during replication in the human gastrointestinal tract, and transcription errors occur with a frequency of 1 per 10^4 bases, approximately one error per genome. As a result, single-site mutations are commonly observed in the 5′ noncoding region of attenuated polioviruses within days after feeding to young infants, and such changes are associated with longer excretion and increased neurovirulence.[40,41] Serial isolates of the same enterovirus serotype excreted over many years by patients with B-cell immunodeficiency syndromes represent a heterogenetic "swarm" with continuous genetic variation, which can be characterized by oligonucleotide fingerprinting[42,43] and RNA sequencing[44] and permits estimation of the duration of infection. In addition, RNA sequencing has led to the discovery of virulent circulating vaccine-derived polioviruses (cVDPVs) that have caused outbreaks of paralytic disease among underimmunized populations in a number of countries previously free of poliomyelitis.[45,46,47,48]

Dual infection with different enteroviral and parechovirus strains may produce recombinant progeny virus if the parent strains are in the same species.[49] Intertypia can be demonstrated in 1 in 10^4 to 1 in 10^5 infectious virions in vitro[50] and in the feces of infants given trivalent OPV. Most cVDPVs isolated to date are OPV viruses that have recombined with the nonpolio serotypes in the enterovirus C species.[46]

Immunity and the Immune Response

Immunity to enteroviral infections is serotype specific. Antibody-mediated immune mechanisms operate in the alimentary tract to prevent mucosal infection and in the blood to prevent dissemination to target organs. Neutralizing antibodies target epitopes primarily located on VP1 and less so on VP2 and VP3.[51] VP1 possesses the largest number of epitopes as a result of being the dominantly exposed viral capsid protein.[8] As early as 1 to 3 days after enteroviral challenge, immunoglobulin M (IgM) humoral antibodies are produced; they predominate in serum during the first month and disappear within 2 to 3 months.[29] IgG antibody, which is typically detected 7 to 10 days after infection, is mostly of the IgG_1 and IgG_3 subtypes.[52] Neutralizing IgG antibodies in serum persist for life after natural infection with enteroviruses.

Small concentrations of humoral type-specific neutralizing antibodies prevented poliovirus viremia and paralysis in experimentally infected primates,[53] and passive immunity to paralytic disease in humans can be achieved by the administration of immune serum globulin before exposure to neurovirulent polioviruses.[54] However, passively administered immune globulin does not modify the outcome of established central nervous system poliovirus disease[55]; at this late stage of infection, patients have detectable serum antibody. There is no proven role for immune globulin treatment of other systemic enterovirus infections.

IgA antibody appears in nasal and alimentary secretions 2 to 4 weeks after the administration of live-attenuated OPV and persists for at least 15 years.[29] However, mucosal immunity is relative: On reexposure to infectious virus, high titers of secretory IgA antibodies prevent or substantially reduce poliovirus shedding, whereas lower titers are associated with more extensive oropharyngeal replication of virus and longer viral shedding.[29] The elaboration of virus-specific IgA antibodies by the small intestine depends on local immunocompetent tissues, as was demonstrated in experiments in infants with double-barrel colostomies who were fed live-attenuated poliovirus through the colostomy and generated secretory IgA antibodies only in the distal loop of the colostomy, not in the pharynx or the proximal loop.[56] Antibodies are present in the colostrum and milk of immune women who are nursing and may interfere with the replication of OPV given to breastfed neonates.[55] Maternal antibodies passively acquired transplacentally or via milk prevent or modify enteroviral infections of early infancy.[57,58] Humoral antibodies have an important role in recovery from enteroviral infection, as evidenced by the development of persistent infections in individuals with significant B-cell immunodeficiency.[59]

There is both clinical and laboratory evidence that humoral antibody alone is not sufficient to limit enteroviral replication in vivo. It has been known that macrophage function is critical for viral clearance and that macrophage ablation enhances the severity of CV-B infections.[60] Host innate immunity appears to play a crucial role in limiting the severity of enteroviral infections. Findings in transgenic mice expressing PVR have shown that the host interferon (IFN) response inhibits poliovirus replication in extraneural tissues and limits the spread of poliovirus in the host.[61] This activity is likely mediated via interaction with Toll-like receptor 3 (TLR3) and the Toll/interleukin-1 receptor domain-containing adaptor-inducing IFN-β pathway.[62–65] The TLR3 pathway appears to have a central role in the immune response to human enteroviral infections as well. TLR3 variants with reduced responsiveness to double-stranded RNA have been associated with enteroviral myocarditis or dilated cardiomyopathy in humans (see Chapter 84).[66] A report examining the role of TLR3 gene polymorphism (TLR3c.1377C/T) in EV-A71 disease severity among Chinese children showed that the TT allele was found more frequently in children with severe rather than mild infection. Children possessing TT allele demonstrated decreased plasma IFN-γ levels and decreased IFN-γ/interleukin-4 ratio compared with children with mild disease.[67]

Inhibition of T-lymphocyte function has little effect on virus replication in vivo,[68] and persons with abnormal cell-mediated immunity are not predisposed to serious or prolonged enterovirus infections unless they have accompanying B-cell dysfunction. Although T lymphocytes do not contribute to the inhibition of enteroviral replication, there is evidence that certain immunopathologic events after enterovirus infection are mediated by T-cell activity. In the murine myocarditis model, expression of proinflammatory cytokines and acute inflammatory infiltration follow peak viral replication,[69–71] and induction of natural killer cell activity and T-lymphocyte immune responses contributes to necrosis of infected cardiac myocytes (see Chapter 84).[68,72,73] An inflammatory response may persist long after viral replication has ceased, and ongoing cardiac damage may be mediated by virus-induced antibodies against cardiac antigens[74] or by cytotoxic T-lymphocyte–mediated myocyte lysis.[70,72,75]

Epidemiology of Enteroviral Infections

Enteroviruses are distributed worldwide. Infection rates vary with season, geography, and age and socioeconomic status of the population sampled. Enteroviral infections occur throughout the year, but in temperate climates infections are more prevalent in the summer and autumn months (June to October in the Northern Hemisphere).[76,77] This seasonal periodicity is less pronounced in southern latitudes and disappears altogether in the tropics, where enteroviruses are endemic the year round. The seasonal pattern of incidence in the United States increased from south to north with the mean occurrence of nonpolio enteroviruses occurring earlier in southern states and northern states.[76] Additionally, the dispersion of cases throughout the year decreased from south to north. Southern states demonstrated a more even distribution of cases within the season, whereas northern states showed an increased peak in distribution.[76]

Age and Socioeconomic Status

The overwhelming majority of enteroviral infections occur in infants, children, and adolescents.[76,77] In the United States, attack rates for both infection and illness with nonpolio enteroviruses are highest in infants during their first year of life.[76,77,78] Surveillance data from 2000–2014 documented that 34% of infections reported to the US Centers for Disease Control and Prevention (CDC) occurred in this age group.[76] Among nearly 26,737 enteroviral detections reported to the CDC for the years 1983–2003, 11.4% occurred in neonates.[77] Rates of symptomatic enteroviral infection decrease after the second month of life[79] but remain higher for infants and toddlers compared with older children and adults.[77,80] Enteroviral infections are more prevalent among children of lower socioeconomic status,[81] probably because of crowding, poor hygiene, and opportunities for fecal contamination. Simultaneous infection by more than one serotype is common under these circumstances.[82]

The frequency with which different enterovirus serotypes cause infection varies markedly. In the United States, one to three enteroviral serotypes predominate in a given location in each season, although there is variation from one region to another and from year to year. Some prevalent serotypes are continuously isolated from year to year,[77] whereas others may emerge for the first time or reemerge after years of relative inactivity.[77,83–85] Wild-type polioviruses now circulate only in three countries (see Chapter 171), whereas vaccine strains are commonly isolated in countries that continue to use OPV for poliomyelitis prevention. Global epidemics of nonpolio enteroviruses occasionally occur, such as the worldwide outbreak of E-9 disease in the late 1950s and the explosive pandemics of acute hemorrhagic conjunctivitis due to EV-C70 and CV-A24 in the 1980s. Infection with some serotypes such as CV-B6 and EV-69 is infrequently or rarely recognized.

The reasons why individual serotypes of enteroviruses appear and disappear and behave as either endemic or epidemic pathogens are not well understood. Some epidemic echovirus strains spread rapidly and exhaust susceptible individuals in the population beyond a critical mass necessary for continued transmission, whereas endemic strains that are recovered over a number of years may be associated with lower attack rates due to a moderate level of herd immunity in the population. Indeed, time-series analysis and mathematical modeling of enterovirus infections in Japan over 15 years supports the theory that the number of susceptible individuals within the population determines when outbreaks occur.[86] In addition, periodic reappearances of the same enteroviral serotype may occur because the new enterovirus strain is poorly neutralized by antibodies raised in response to earlier strains.[87–89] Over the 11-year period between 2006 and 2016, 14 serotypes represented 81% of all enterovirus isolates submitted from state and local public health laboratories to the National Enterovirus Surveillance System of the CDC (Table 170.4).[90–92]

Molecular Epidemiology

Southern blotting, two-dimensional oligonucleotide gel RNA electrophoresis, and amplification and sequencing of defined RNA sequences have been used to differentiate between live vaccine and naturally occurring poliovirus strains[93,94] and trace the routes of spread of polioviruses[95] and other enteroviruses by comparison of RNA relatedness among epidemiologically distinct isolates.[96–98] Genomic RNA sequencing has proved to be the most adept technique for characterizing the evolutionary relationships among poliovirus isolates of the same serotype. Genotypes are distinguished from one another by greater than 15% divergence among the RNA nucleotides in the homologous portions of the genomes that are sequenced.[94] The application of genomic sequencing has detected cVDPVs in Haiti and other locations[45,46]; traced the pandemic spread of acute hemorrhagic conjunctivitis caused by EV-D70 and CV-A24 in the 1980s[99]; and documented the rise of new clades of EV-D68, a cause of severe respiratory infection.[83,100]

TABLE 170.4 Most Common Enterovirus Serotypes Submitted by State and Local Public Health Laboratories to the Centers for Disease Control and Prevention, 2006–2016

ENTEROVIRUS SEROTYPE	% OF ENTEROVIRUS ISOLATES
Enterovirus D-68	28.1
Coxsackievirus A6	6.6
Echovirus 18	5.6
Echovirus 30	5.3
Echovirus 11	4.8
Echovirus 9	4.6
Coxsackievirus B1	4.5
Echovirus 6	4.5
Coxsackievirus B4	3.9
Coxsackievirus A9	3.8
Coxsackievirus B3	3.2
Coxsackievirus B5	3.1
Coxsackievirus A16	1.7
Coxsackievirus B2	1.6
Total	81.3

Data from references 90–92.

Transmission

Enteroviruses are shed in feces and respiratory tract secretions. Transmission occurs from direct person-to-person contact and indirectly from environmental sources contaminated by enteroviruses. The relative importance of each route of transmission depends on viral factors, hygienic conditions, and, possibly, climatic conditions. Computational analysis of the seasonality of enteroviral infections in the United States indicates that dew point temperature was a main predictor of intensity of transmission.[76] Direct transmission from a respiratory source has been demonstrated in volunteer studies using CV-A21 as a challenge virus,[101] and it is likely that other enteroviruses frequently shed simultaneously from the upper respiratory tract and feces are also transmitted in this manner.[102] However, fecal contamination of the household environment is responsible for the high rates of infection demonstrated with polioviruses and many other enterically shed enteroviruses in poor hygienic conditions. Sampling of sewage in most cities, especially in summer months, usually yields several enteroviral serotypes.[103] Clams in seawater polluted by sewage concentrate enteroviruses 10- to 60-fold, and swimming in contaminated seawater was the apparent cause of an outbreak of enterovirus infection among tourists returning from Mexico.[104] Enterovirus D70, the agent of acute hemorrhagic conjunctivitis, is spread by fomites, fingers, and ophthalmologic instruments contaminated with virus in tears.[105]

Longitudinal studies have shown substantial clustering of enterovirus infections in families.[102] Once the virus has been introduced into the household, secondary attack rates for infection among seronegative family members are 90% to 100% for wild-type polioviruses and approximately 75% for coxsackieviruses.[102] Secondary attack rates for echoviruses range from less than 50% to 80%,[106] probably because these viruses tend to be shed only in feces and for shorter periods. Infants

in diapers who shed virus in the feces are the most efficient disseminators of infection. Mothers and infant siblings are at greater risk of acquiring infection than are fathers and teenaged siblings.[102] For all enteroviruses, the period of maximal contagiousness corresponds to the period of maximal viral excretion in the feces. When reinfection with the same enterovirus serotype occurs, the duration of excretion of virus is considerably shorter than in the primary infection.[102]

Incidence of Infection and Illness

Approximately 95% of infections caused by wild-type polioviruses and at least 50% to 80% of nonpolio enteroviral infections are asymptomatic. When illness occurs, it usually takes the form of an undifferentiated febrile illness lasting only a few days, often accompanied by symptoms of upper respiratory tract infection.[107] These illnesses may be caused by virtually any enteroviral serotype and are clinically indistinguishable from infection by many other viruses. Disease syndromes characteristic of enteroviruses (e.g., aseptic meningitis, pericarditis) are infrequent manifestations of infection. A 4-year longitudinal family-based study in New York City detected 291 enteroviral infections, none producing characteristic illnesses and only 6 with exanthems.[102]

The risk of some enterovirus-related clinical syndromes varies with age and sex. Aseptic meningitis is most commonly recognized in very young infants, whereas some other illnesses such as pleurodynia and myopericarditis are seen predominantly in adolescents and young adults. Symptomatic enteroviral infections in elderly adults are uncommon. Among young children, boys are at greater risk of illness (but not infection) than girls.[77,78] Aseptic meningitis and poliomyelitis occur almost twice as often in boys. After puberty, the reverse is true, perhaps because women have greater exposure than men to children shedding virus.[102,108] Pregnancy may enhance the severity of enteroviral infections. The incidence of paralytic poliomyelitis was two to three times higher in pregnant women than in age-matched nonpregnant women in Boston before the control of poliomyelitis.[109] Also, clinical and epidemiologic data demonstrate that enteroviral illnesses are more frequent[110] and more severe[111] in persons who exercise vigorously before the onset of symptoms. Although these data are anecdotal, they are supported by evidence that exercise enhances the severity of CV-B infection in the murine model.[112]

Although the incidence of nonpolio enteroviral disease has been measured in selected populations, the overall incidence in the United States is unknown. Serologic surveys encompassing all known enteroviral serotypes are not feasible. Antibody prevalence rates measured for a few serotypes indicate that following the decline of passively acquired maternal antibodies after the age of 6 months, the percentage of immune persons in the population rises progressively with age, so that 15% to 90% of the adult population have type-specific neutralizing antibodies for each serotype tested, depending on the serotype and the characteristics of the population surveyed.[78,102]

Incubation Period and Period of Communicability

The incubation period for illness due to enteroviral infections can rarely be determined precisely. Because the source of infection is often an asymptomatic person who transmits virus as readily as one who is ill, the time of exposure is usually unknown. Although the incubation period may range from 2 days to 2 weeks, it is usually 3 to 5 days. Patients with enteroviral illnesses typically excrete virus in throat secretions or feces for several days before the onset of symptoms and continue to excrete virus in feces for several weeks thereafter. Although the period of communicability is potentially long, maximal communicability probably occurs early in illness, when viral shedding is greatest.

Laboratory Diagnosis of Enteroviral Infections

The laboratory diagnosis of enteroviral infection is accomplished by virus isolation in cell culture, nucleic acid amplification testing (NAAT) (i.e., reverse-transcriptase polymerase chain reaction [RT-PCR] or nucleic acid sequence-based amplification), or retrospectively by serologic methods.[113] Cell culture is still performed by some academic medical centers and public health laboratories. However, because cell culture is laborious and relatively slow, it has been supplanted by more rapid and sensitive NAAT.

Viral Isolation

A presumptive diagnosis of enteroviral infection can usually be reported by the laboratory within 2 to 5 days by identification of a characteristic cytopathic effect in any of three or four appropriately chosen cell lines or cell line mixtures.[113,114] CV-As grow poorly in routinely used cell lines. Inoculation of newborn mice remains the method of choice for their recovery but is rarely used today because of its laborious nature.[113]

Isolates demonstrating a cytopathic effect may be preliminarily screened for enterovirus species or serotype with the use of monoclonal antibody blends.[113] Traditionally, the serotype of an enterovirus isolated in cell culture was determined by neutralization in cell culture using type-specific antisera. However, this has been largely replaced by molecular methods based on sequencing of the VP1 coding region.[113] Serotype identification by neutralization requires the use of Lim-Benyesh-Melnick intersecting antiserum pools.[115,116] The equine sera that constitute the Lim-Benyesh-Melnick pools were harvested against enterovirus strains prevalent more than 40 years ago, which explains their diminishing ability to identify contemporary isolates as well as their failure to identify newer human enteroviruses.

The opportunity to recover a virus in cell culture is optimized by sampling of multiple sites. Late in the course of enteroviral illnesses, viral cultures of feces are useful because the lower intestine may be the only site from which the agent is still being excreted. An etiologic diagnosis can be confirmed by the isolation of virus from cerebrospinal fluid, pericardial fluid, tissue, or blood, depending on the clinical syndrome. Isolation of virus from the upper respiratory tract or stool is considered to be less definitive because intercurrent asymptomatic enterovirus infections etiologically unrelated to the observed illness may produce a false-positive result.

Nucleic Acid Amplification Testing and Genomic Sequencing

RT-PCR and nucleic acid sequence-based amplification are rapid, sensitive, and specific methods of detecting enterovirus RNA in clinical specimens.[113] Most reported NAAT protocols amplify a highly conserved portion of the 5′ nontranslated region of the genome, enabling the detection of most enteroviruses.[117] Group, serotype, vaccine, and vaccine-related specific primers distinguish naturally occurring vaccine and vaccine-derived polioviruses from other enteroviruses.[118–120] With cerebrospinal fluid specimens from patients with suspected aseptic meningitis, NAAT has been shown to be superior to cell culture for the detection of enteroviruses, increasing yields from 7% to 61% in various studies.[113,117] Experience with specimens other than cerebrospinal fluid is more limited. NAAT has detected enteroviral RNA from throat swabs, serum, myocardium, urine, and stool, although the sensitivity with urine specimens is lower than with other specimens.[113,117,121,122] NAAT detected enteroviral RNA in approximately 15% of endomyocardial biopsy specimens from patients with acute myocarditis or dilated cardiomyopathy.[123] Further characterization of the species and serotype of enteroviruses isolated in cell culture or detected by RT-PCR can be accomplished by sequencing a portion of the VP1 capsid coding region of the viral genome.[2,3,123,124]

Serology

Type-specific, homotypic, and heterotypic immunoassays have been developed for the serologic diagnosis of enterovirus infections.[125–129] However, the utility of these assays as clinically relevant tools is limited by a number of issues. Serum IgM antibody to the enteroviruses may be nonspecific, leading to false-positive results, cross-reactivity with other *Picornaviridae,* and less sensitivity than with NAAT. Attempts to improve the sensitivity of the serologic assays have led to their enhancement using RT-PCR.[128] However, the latter are generally technically challenging to implement. Until identification of a common antigen sufficiently immunogenic to form the basis of a broadly reactive assay, serologic diagnosis will have limited use in the clinical management of enteroviral infections.

Treatment and Prevention of Enteroviral Infections

Most enterovirus infections are self-limited and do not require antiviral therapy. Exceptions include encephalitis, acute myocarditis, and infections in neonates and B-cell–deficient hosts that may be life threatening. The therapeutic options for these more serious infections are quite limited. Serum immune globulin and intravenous immune globulin have been given to persistently infected B-cell–deficient patients with mixed results[59] and have been used in nonrandomized trials in children with myocarditis and neonates with uncertain effect.[130,131]

An effective antiviral drug to treat serious enterovirus infections has not been licensed. The most promising of the compounds to undergo clinical trials has been pleconaril, an antiviral that binds to a pocket in the viral capsid, altering virus attachment and uncoating.[132,133] Pleconaril was found to inhibit replication of most enterovirus serotypes at concentrations of less than 0.1 μg/mL in vitro.[133] In a multicenter, placebo-controlled study, neonates with enteroviral sepsis (hepatitis, coagulopathy, or myocarditis) treated with pleconaril had greater survival and shorter times to PCR and culture negativity than infants who received placebo.[134] A post hoc analysis of two multicenter, double-blind, placebo-controlled trials of pleconaril in adults with enterovirus meningitis documented reduction in the duration of headache for patients with moderate to severe nausea.[135] Several additional non–peer-reviewed presentations of the results from pleconaril clinical trials also indicated benefit.[136] Uncontrolled experience with pleconaril for B-cell–deficient patients with persistent enterovirus infections and patients with potentially fatal infections including neonates and patients of all ages with acute myocarditis suggests possible clinical benefit.[137,138] Pleconaril has also shown mild benefit in the treatment of rhinovirus-induced colds,[139] but it was not approved by the US Food and Drug Administration for this indication. The drug induces cytochrome P-450 3A isoenzymes[139] and therefore has raised concern for multiple drug interactions.[138] Pleconaril is currently unavailable for clinical use (see Chapter 48).

The preexposure administration of immune globulin is known to reduce the risk of paralytic poliomyelitis.[54] It is likely that immune globulin would also prevent nonpolio enteroviral disease, but this strategy is rarely applicable to clinical practice. The successful vaccine approach against paralytic poliomyelitis is detailed in Chapter 171. In the setting of a community epidemic or a patient hospitalized with enteroviral illness, simple hygienic measures such as hand washing[140] and careful disposal or autoclaving of potentially infected feces and secretions should be practiced. For hospitalized patients, infection control measures using standard precautions are sufficient. Gown and mask procedures or isolation of the patient is not warranted except in the newborn nursery. In nursery outbreaks of enteroviral infection, infected infants should be cohorted. Pregnant women, especially those near term, should be advised to avoid contact with people suspected to have an enteroviral illness.

PARECHOVIRUSES

The human parechoviruses (HPeV) have many properties in common with other picornaviruses, but they also have biochemical and biophysical differences that have led to their designation as a separate genus of *Picornaviridae*.[2] The properties of HPeV are reviewed in the following paragraphs and discussed in more detail in Chapter 173.

Virology

Parechoviruses have the typical morphology of picornaviruses. They are small (28 nm), are nonenveloped, and have icosahedral symmetry. The capsid consists of 12 pentamers. Virions have a buoyant density in 1.34 g/cm of cesium chloride.[2,8,141] The genome of HPeVs is contained in a nonsegmented, linear, single-stranded RNA of positive sense. The complete genome is approximately 7350 nucleotides long, the organization of which is similar to the human enteroviruses: a 5′ nontranslated region of approximately 740 nucleotides, a coding region of approximately 6540 nucleotides, a short 3′ nontranslated region, and a 3′ polyadenylated tail.[141]

The genome encodes a single polyprotein, which undergoes cleavage into three capsid proteins (VP0, VP1, VP4) and seven nonstructural proteins and protein intermediates. However, in contrast to enteroviruses, cleavage of VP0 into VP4 and VP2 does not occur, resulting in the parechoviruses having only three structural proteins: VP0, VP1, and VP3.[8,141] The nonstructural proteins include an RNA-dependent RNA polymerase, VPg, and a probable helicase.[141] Receptors for HPeV1 appear to be the $\alpha_v\beta_1$, $\alpha_v\beta_3$ integrins (see Table 170.3).[8,142]

Virus Types

Because HPeVs replicate poorly in cell culture and owing to the relatively laborious and difficult process of determining neutralization of parechoviruses in tissue culture, sequencing of the VP1 capsid coding region is employed to designate types. There have been 17 types identified.[4]

Viral Diagnosis

Commercially available NAAT is available for detection of the HPeV. Serologic responses are infrequently used, but determination of virus-specific IgG or IgM neutralizing responses is occasionally used to establish the occurrence of infection.[141]

Epidemiology

HPeV1 (formerly known as echovirus 22) appears to be widely distributed and mainly infects young children. Infection with HPeV2 (formerly echovirus 23) appears to be much more limited.[141] HPeV3 is found worldwide and is the major cause of central nervous system infections due to the human parechoviruses. Infections occur primarily in infants younger than 6 months of age.[143] The epidemiology and clinical manifestations of parechovirus infections are discussed in Chapter 173.

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The complete reference list is available online at Expert Consult.

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171 Poliovirus

José R. Romero

SHORT VIEW SUMMARY

Definition

- Polioviruses are the cause of poliomyelitis, a systemic viral infection that predominantly affects the central nervous system, causing paralysis. Only three serotypes exist (1 to 3). The name of the disease (*polios,* "gray"; *myelos,* "marrow" or "spinal cord"), now commonly shortened to polio, is descriptive of the pathologic lesions that involve neurons in the gray matter, especially in the anterior horns of the spinal cord.

Epidemiology

- Wild-type poliovirus type 2 no longer circulates worldwide and was declared eradicated in 2015. Serotype 3 has not been isolated since November 2012. Serotype 1 remains endemic in three countries (Afghanistan, Nigeria, and Pakistan). In 2017, the annual number of cases of poliomyelitis (19) was the lowest since the initiation of the global poliovirus eradication effort in 1988.

Microbiology

- The polioviruses are members of the genus *Enterovirus,* family Picornaviridae. Morphologically, they are small (30 nm in diameter), nonenveloped, icosahedral-shaped viruses that possess a single-stranded, positive-sense RNA genome.

Diagnosis

- Polioviruses can be isolated from throat secretions and from feces and rarely from the cerebrospinal fluid. Characterization is accomplished by sequencing of the major capsid protein VP1 and is available only in public health reference laboratories.

Therapy

- No specific therapy is available. Supportive measures to ensure airway patency, adequate respiratory effort, and clearance of secretions are the mainstay of treatment for severe cases of paralysis.

Prevention

- Vaccination with inactivated or live-attenuated oral vaccines has been essential in the elimination of the polioviruses. In preparation for the global eradication of the polioviruses, a worldwide switch to bivalent oral polio vaccine containing poliovirus types 1 and 3 occurred in 2016. Public health measures, such as provision of potable water and proper sewage disposal, play major roles in the community-wide control of the polioviruses.

Polioviruses are the cause of poliomyelitis, a systemic viral infection that predominantly affects the central nervous system (CNS), causing paralysis. The name of the disease (*polios,* "gray"; *myelos,* "marrow" or "spinal cord"), now commonly shortened to polio, is descriptive of the pathologic lesions that involve neurons in the gray matter, especially in the anterior horns of the spinal cord. Paralytic poliomyelitis has been completely controlled in the United States and other developed countries[1] through routine childhood immunization with either inactivated poliovirus vaccine (IPV), live-attenuated oral poliovirus vaccine (OPV), or both. However, as of 2017,[1] the goal of worldwide poliomyelitis eradication has not yet been achieved, having been hampered by unforeseen scientific challenges, geopolitical unrest, and social disruption, among other factors.

HISTORY

Perhaps the earliest representation of poliomyelitis is found in a funerary stele from the Ramesside period (1300 BC) of Ancient Egypt, housed in the Carlsberg Museum, Copenhagen. It depicts the priest Rom with an atrophic lower extremity and foot held in a talipes equinus position.[2,3] Sporadic poliomyelitis cases were published as early as 1840, and the first descriptions of the natural history and neurologic complications of poliomyelitis were recorded in Sweden by Karl Oskar Medin in 1890.[4] There is little record of epidemic poliomyelitis until the late 19th century, when outbreaks were first recorded in Scandinavia, Western Europe, and the United States. Charles Caverly wrote the first description of epidemic poliomyelitis in the United States, an outbreak of 132 cases near Rutland, Vermont, in 1894.[5] Thereafter, sporadic epidemic disease occurred during the first half of the 20th century, and by the 1950s, epidemic polio occurred regularly, with approximately 25 cases per 100,000 population reported annually in the United States. Accompanying the increased incidence was a shift in the peak-affected age group from infants to school-aged children and young adults. Both the appearance of epidemic disease and the rising age incidence have been attributed to rising standards of hygiene, which delayed the age of infection beyond infancy and loss of maternal antibody, thus creating a pool of susceptible people large enough to permit the spread of epidemic disease.[6]

In 1908, Landsteiner and Popper[7] demonstrated that polio was caused by a "filterable virus" when they transmitted disease to monkeys from human spinal cord homogenates. Scientific progress remained somewhat limited until the landmark discovery in 1949 by Enders, Weller, and Robbins[8] that poliovirus could be propagated in vitro in cultures of human embryonic cells of nonneural origin. This discovery facilitated experimental investigation of the pathogenesis of the disease in primates and the development of vaccines. Bodian and associates[9] first recognized the three distinct serotypes of poliovirus. By 1952, Bodian[10] and Horstmann[11] had independently discovered that viremia occurred early in infection, which explained the systemic phase of the illness.

Salk[12] reported in 1953 that human subjects could be successfully immunized with formalin-inactivated poliovirus, a discovery that rapidly led to an extensive field trial and licensure of IPV in 1955. The introduction of IPV led to a sudden dramatic reduction in the incidence of paralytic poliomyelitis in the United States to 0.4 cases per 100,000 population in 1962, when OPV replaced IPV for routine use. The last case of endemic wild-type poliomyelitis occurred in 1979, indicating complete cessation of transmission of naturally occurring polioviruses.[13] However, in addition to rare cases of wild-type disease imported from other countries, the United States continued to experience 6 to 10 cases of OPV vaccine-associated paralytic poliomyelitis (VAPP) each year until 1997, when IPV was reintroduced into the routine childhood immunization schedule in combination with OPV. Since 2000, only IPV has been used.

Under the leadership of the Pan American Health Organization, the entire Western Hemisphere became free of paralytic polio by 1991. Poliovirus type 2 was certified as globally eradicated in 2015. As of the end of 2017, worldwide, endemic circulation of polioviruses had been controlled by all countries with the exception of Pakistan, Afghanistan, and Nigeria.[1] No naturally occurring polioviruses have been identified from any other country since 2014.[1] Virulent polioviruses derived from OPV strains (circulating vaccine-derived polioviruses [cVDPVs]) also cause periodic outbreaks of paralytic polio in some regions with low population immunity.[14]

PATHOPHYSIOLOGY

Virology

Polioviruses are prototypic members of the genus *Enterovirus*[15] (see Chapter 171). Three poliovirus serotypes exist, and infection with each confers serotype-specific, lifelong immunity to disease but little or no immunity to infection or disease caused by heterologous serotypes.[16] Before the introduction of poliovirus vaccines, most paralytic disease was caused by type 1.[17] Polioviruses are categorized into three groups: wild-type (naturally occurring) polioviruses (WPVs), vaccine-related poliovirus (VRPV) strains (live-attenuated OPV viruses), and vaccine-derived polioviruses (VDPVs) arising from OPV strains. The VDPVs are further classified as cVDPV when evidence of person-to-person transmission exists; immunodeficiency-associated VDPV (iVDPV) when isolated from persons with primary immunodeficiencies; and ambiguous VDPV (aVDPV) when isolated from individuals without immunodeficiency and evidence of transmission, or in the case of sewage isolates unrelated to other known VDPVs and whose source is unknown.[14,18] WPV, VPDV and VDPD may be encountered in different populations, depending on whether endemic transmission of WPV has been eliminated, on whether OPV is used, and on the vaccine-induced immunity rates in the population.

Humans are the only natural host and reservoir of polioviruses. Experimental infections and paralysis can be produced in other primates, polioviruses can be adapted to replicate in subprimate mammals, and transgenic (tg) mice expressing the poliovirus receptor (PVR) can be paralyzed by injection into the CNS or peripheral muscle but not by the oral route.[19] Recently, tg mice expressing PVR under the control of the murine promotor Tage4 have been demonstrated to be moderately susceptible to oral infection by poliovirus.[20] Naturally occurring strains vary over a 10^7-fold range in neurovirulence.[21] Polioviruses are more infectious for the human gut than for the gut of lower primates.

Attenuated OPV strains are occasionally able to paralyze rhesus and cynomolgus monkeys but only when injected in high doses directly into the CNS. In addition to low neurovirulence, vaccine strains can often be distinguished from WPV strains by their temperature sensitivity and by subtle antigenic differences. The RNA sequences of the vaccine strains differ from the sequences of their naturally occurring parents by less than 0.2% across the full genome, with the smallest difference occurring between the type 2 vaccine and parent strains. For all three serotypes, analogous nucleotide substitutions in the 5′-noncoding region are associated with reduced neurovirulence.[22–25] Attenuating mutations also map to capsid proteins for individual serotypes.

In contrast, cVDPVs have been permitted to circulate because of low population immunity for long periods of time and, by continuous mutation, acquire biologic properties indistinguishable from those of WPVs.[14,17,26] The cVDPVs isolated have RNA sequences that vary by more than 0.6% to 1.0% from the corresponding OPV parent strain within the region encoded for the VP1 capsid protein, have undergone genomic recombination with other C species enteroviruses, and have acquired virulence markers characteristic of WPV strains.[18]

Pathogenesis

The early events in the pathogenesis of poliomyelitis are similar to those in other enterovirus infections described in Chapter 171. After implantation at a mucosal site and replication in the gut, or less frequently the throat and adjacent lymphoid tissues, polioviruses disseminate to susceptible reticuloendothelial tissues via a minor viremia. In asymptomatic infections, the virus is contained at this point and elicits the formation of type-specific antibodies. In a few infected persons, replication in the reticuloendothelial system gives rise to a major viremia, which corresponds temporally with the minor illness and causes the symptoms associated with abortive poliomyelitis. At this point, the course of poliomyelitis deviates from that of other enteroviral diseases in the ability of polioviruses to infect neurons in the gray matter of the brain and spinal cord. Although the preponderance of evidence indicates that viremia precedes paralysis in both experimental primates and humans, the exact routes whereby the CNS becomes infected remain uncertain.[27,28] Studies in the PVR tg mouse suggest that polioviruses may enter the CNS via the bloodstream or by retrograde axonal transport in peripheral nerve fibers.[29–31] Neuropathologic studies and animal experiments have indicated that spread is neural once the virus reaches the CNS.[32,33]

Poliovirus principally affects motor and autonomic neurons. Neuronal destruction is accompanied by inflammatory lesions consisting of polymorphonuclear leukocytes, lymphocytes, and macrophages that are distributed throughout the gray matter of the anterior horns of the spinal cord and the motor nuclei of the pons and medulla.[32,34] The mesencephalon, cerebellar roof nuclei, and precentral gyrus of the cerebral cortex are less severely involved.[34] Clinical symptoms depend on the severity of lesions rather than on their distribution, which is similar in essentially all cases; almost all fatal cases have involvement of both the spinal cord and cranial nerve nuclei and brainstem, even in the absence of bulbar signs. The dorsal root ganglia are commonly involved pathologically, but this does not result in sensory deficits. Polioviruses can be isolated from the spinal cord for only the first few days after the onset of paralysis, but the inflammatory lesions may persist for months.

CLINICAL MANIFESTATIONS

Incubation Period

Best estimates of the incubation period of poliomyelitis are 9 to 12 days (range, 5–35 days), measured from presumed contact until the onset of the prodromal symptoms, and 11 to 17 days (range, 8–36 days) until the onset of paralysis.[35]

Clinical Manifestations of Infection

The manifestations of infection by polioviruses range from unapparent illness to severe paralysis and death. Usual estimates of the ratio of unapparent to clinically recognized polio infection, which vary by serotype, range from 60:1 to 1000:1.[6,36] Fig. 171.1 depicts the time

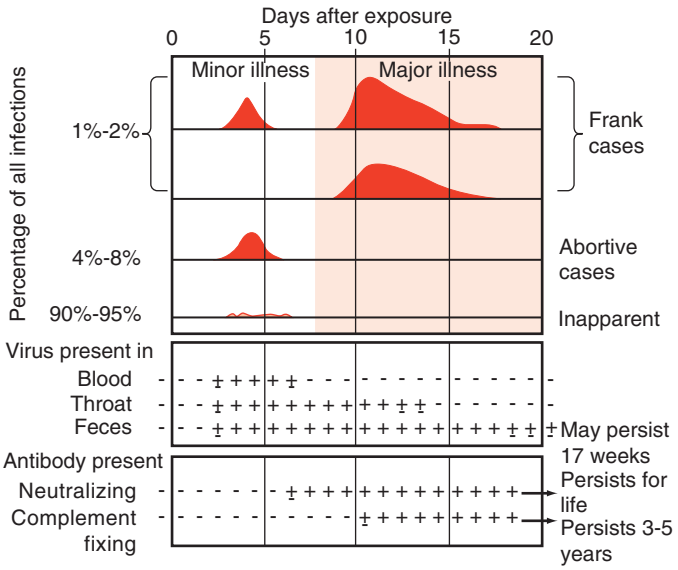

FIG. 171.1 Schema of the clinical and subclinical forms of poliomyelitis. This graphic representation shows the presence of virus and antibodies in relation to the development and persistence of the infection. *(From Paul JR.* History of Poliomyelitis. *New Haven, CT: Yale University Press; 1971.)*

course for the clinical manifestations of poliovirus infection. At least 95% of infections are asymptomatic or unapparent and can be recognized only by the isolation of poliovirus from feces or oropharynx or by a rise in antibody titer. *Abortive poliomyelitis,* which occurs in 4% to 8% of infections, is characterized by a 2- to 3-day period of fever, which may be accompanied by headache, sore throat, listlessness, anorexia, vomiting, or abdominal pain. Because the neurologic examination findings are normal, abortive poliomyelitis cannot be distinguished from other viral infections and can be clinically suspected only during an epidemic. *Nonparalytic poliomyelitis* differs from abortive poliomyelitis by the presence of signs of meningeal irritation. The disease is identical to meningitis caused by other enteroviruses. The systemic manifestations of nonparalytic poliomyelitis are generally more severe than in abortive poliomyelitis.

Spinal Paralytic Poliomyelitis

Frank paralysis occurs in roughly 0.1% of all poliovirus infections. A biphasic course with minor and major illnesses is observed in approximately one-third of children with paralytic poliomyelitis. The minor illness, coinciding with viremia, corresponds to the symptoms of abortive poliomyelitis and lasts 1 to 3 days. The patient appears to be recovering and remains symptom free for 2 to 5 days before the abrupt onset of the major illness, which is heralded by symptoms and signs of meningitis, including fever, chills, headache, fever, malaise, vomiting, neck stiffness, and cerebrospinal fluid (CSF) pleocytosis. Adults usually experience a single phase of illness, with a prolonged prodrome of symptoms preceding the gradual onset of paralysis.[37,38] The major illness begins with severe myalgias and occasionally localized cutaneous hyperesthesia, paresthesias, involuntary muscle spasm, or muscular fasciculations. The meningismus and muscle pain are present for 1 to 2 days before frank weakness and paralysis ensue. The severity of the disease varies from weakness of a single portion of one muscle to complete quadriplegia. The paralysis is flaccid; deep tendon reflexes are initially hyperactive and then become absent. The most characteristic feature of the paralysis is its asymmetrical distribution, which affects some muscle groups while sparing others. Proximal muscles of the extremities tend to be more involved than distal muscles, the legs are more commonly involved than the arms, and the large muscle groups of the hand are at greater risk than the small ones. Any combination of limbs may be paralyzed, but the most common pattern is involvement of one leg, followed by one arm, or both legs and both arms. Quadriplegia is almost never observed in infants.[38] Although occasional cases progress from the onset of weakness to complete quadriplegia and bulbar involvement in a few hours, more commonly the paralysis extends over 2 to 3 days. Progression of paralysis stops when the patient becomes afebrile.[37] Paralysis of the bladder is usually associated with paralysis of the legs. It occurs in about 25% of adults but is uncommon in children. Sensory loss in poliomyelitis is very rare,[39] and its occurrence should strongly suggest some other diagnosis (e.g., Guillain-Barré syndrome).

Bulbar Paralytic Poliomyelitis

Bulbar poliomyelitis results from paralysis of muscle groups innervated by the cranial nerves, especially those of the soft palate and pharynx, which may manifest as dysphagia, nasal speech, and sometimes dyspnea. The frequency of the bulbar form of the disease has varied in different epidemics from 5% to 35% of paralytic cases. The ninth and tenth cranial nerves are most commonly involved, and pharyngeal paralysis with pooling of secretions often is the only obvious sign.[40] Patients usually are extremely anxious and agitated about their inability to swallow and breathe. Involvement of the circulatory and respiratory centers of the medulla represents the most serious form of bulbar poliomyelitis.

Polioencephalitis

Encephalitis, manifesting primarily with confusion and disturbances of consciousness, is an uncommon form of poliomyelitis occurring principally in infants. It is the only type of poliomyelitis in which seizures are common. In contrast to spinal paralytic polio, there may be spastic paralysis, indicating the presence of upper motor neuron involvement. The illness is not clinically distinguishable from other forms of viral encephalitis.

Complications

The most important complication of paralytic poliomyelitis is respiratory compromise caused by paralysis of the respiratory muscles, including the diaphragm and intercostal muscles, by airway obstruction from involvement of the cranial nerve nuclei, or by lesions of the medullary respiratory center.[41] Myocarditis has been documented by the presence of inflammatory infiltrates in cardiac tissue obtained postmortem and isolation of poliovirus from myocardium,[42,43] but myocardial dysfunction is rarely recognized clinically. Gastrointestinal events such as hemorrhage, paralytic ileus, and gastric dilatation may complicate acute paralysis.[44] Paresis of the detrusor muscle can lead to bladder paralysis and urinary retention in the acute phase of paralytic poliomyelitis.[45,46] Patients with prolonged recumbency are at risk for the development of renal calculi.

RISK FACTORS

Several preexisting factors and provocative events are known to influence the risk of poliovirus infection and, after infection with poliovirus, the risk of developing paralysis. Before puberty, poliovirus infections occur equally in boys and girls, although paralysis is more common in boys. Among adults, women are at greater risk of infection but are not necessarily at greater risk of paralysis.[47,48] Both the incidence and severity of poliomyelitis may be increased in pregnant women.[48,49] Not only are women of childbearing age more likely to be exposed to infections in young children,[47] but also, late pregnancy may be associated with increased susceptibility to more serious illness.

Strenuous exercise increases both incidence and severity of paralytic poliomyelitis.[50,50a] Exercise during the minor illness or the prodrome has no effect but is detrimental when it occurs during the first 3 days of the major illness.[50]

Both epidemiologic[51,52] and experimental[53,54] studies have confirmed that poliomyelitis tends to localize in a limb that has been the site of an intramuscular injection or injury within 2 to 4 weeks before the onset of infection. Provocation poliomyelitis has been observed with both wild-type and OPV viruses.[55] Skeletal muscle injury in viremic PVR-expressing tg mice induces retrograde axonal poliovirus transport to the CNS.[54]

Tonsillectomy increases the risk of bulbar poliomyelitis whether the procedure is performed just before poliovirus infection or was performed in the remote past.[56,57] Because the ninth and tenth cranial nerves supply the fauces, the spread of virus from damaged nerve endings may explain this effect.

DIFFERENTIAL DIAGNOSIS

Sporadic cases of acute motor neuron disease that is indistinguishable from poliomyelitis may be caused by other enteroviruses, especially enteroviruses A-71 and D-68 (see Chapter 172) or West Nile virus infection[58] (see Chapter 153). Few other diseases are confused with paralytic poliomyelitis, except Guillain-Barré syndrome, which, unlike poliomyelitis, produces symmetrical, bilateral ascending paralysis, with loss of sensation that in most cases may progress over a period of 1 to 2 weeks. In acute poliomyelitis, pleocytosis and a minimally elevated protein concentration are present, whereas in Guillain-Barré syndrome the protein level is elevated with absent or minimal pleocytosis (the albuminocytologic dissociation).

LABORATORY DIAGNOSIS

Abnormalities of the CSF are not distinguishable from those of other viral diseases that cause aseptic meningitis. Polioviruses usually can be isolated from throat secretions in the first week of illness and from feces, often for several weeks. Unlike other enteroviruses that cause aseptic meningitis, polioviruses rarely are isolated from the CSF. In sporadic cases of poliomyelitis occurring in areas of low incidence, it is important to characterize virus isolates as wild-type (naturally occurring strains), OPV virus, or cVDPVs that have evolved from OPV virus. In all cases, this is accomplished by genomic sequencing, available only in public health reference laboratories.[59,60] Isolation from CSF (or brain and spinal cord in fatal cases), although uncommon, is especially valuable in evaluating VAPP, because recovery of fecal virus is expected for several weeks after receipt of OPV, and only a CNS virus isolate with

vaccine characteristics provides conclusive evidence of the causative association.

In the absence of a viral isolate, the diagnosis of poliovirus infection can be established serologically by testing paired acute and convalescent sera for neutralizing antibodies to each of the three poliovirus serotypes. Serologic tests cannot distinguish between wild-type virus and vaccine virus infection.

PROGNOSIS

Muscle paralysis usually progresses or extends for only 1 to 3 days after its onset but occasionally for as long as 1 week.[61] Permanent weakness is observed in approximately two-thirds of patients with paralytic poliomyelitis. Complete recovery is less likely when acute paralysis is severe and when patients require mechanical ventilation. An estimate of the eventual outcome can be made after 1 month, when most reversible damage has disappeared. Very little additional return of function can be expected beyond 9 months. Recovery from pharyngeal paralysis usually is evident by 10 days and is eventually complete. Bulbar poliomyelitis is rarely responsible for permanent sequelae in surviving patients.

Available mortality figures date from the era of epidemic poliomyelitis, a period when critical care medicine was less advanced than it is today. The reported overall mortality for acute paralytic poliomyelitis during this period was approximately 5% to 10%.[62] Mortality rates are higher among older individuals, who are more likely to have combined spinal respiratory muscle and bulbar involvement.[62,63] A poliovirus type 1 outbreak in the Republic of the Congo was associated with a case fatality rate of 32% and 48% in persons younger than 15 and those 15 years of age and older, respectively.[64]

THERAPY

Although antiviral drugs for the treatment of poliovirus-infected persons are under development,[65,66] they are not yet available, and therefore management is supportive and directed at relief of symptoms.[67] The most advanced antiviral in terms of clinical study is pocapavir (V-073), a capsid inhibitor with in vitro activity against polioviruses.[68,69] It was studied in a placebo-controlled challenge of 144 volunteers who received oral poliovirus type 1 vaccine, where it accelerated clearance of virus but was associated with frequent emergence of virus resistant to pocapavir.[66] Antivirals against polioviruses and other enteroviruses are further discussed in Chapter 48. In the acute phase of paralytic poliomyelitis, patients should be hospitalized. Bed rest may prevent augmentation or extension of paralysis. Hot moist packs applied to muscles are helpful for relieving pain and spasm. Physical therapy should be initiated once the progression of paralysis has ceased.

Paralysis of the respiratory muscles necessitates mechanical ventilation before hypoxia develops, generally when the vital capacity falls to less than 50%. Pooling of secretions in the pharynx in mild bulbar poliomyelitis, if unaccompanied by spinal respiratory paralysis, can be managed with postural drainage and suction. Severe bulbar paralysis necessitates tracheal intubation. Weakness or paralysis of the bladder may necessitate catheterization.

Management of the long-term physical and psychiatric sequelae of paralytic poliomyelitis is beyond the scope of this text. The reader is referred to excellent references on these topics.[70–72]

POSTPOLIOMYELITIS SYNDROME

Some patients who partially or fully recover from paralytic poliomyelitis experience a new onset of muscle weakness, pain, atrophy, and fatigue many years after the acute illness.[73] Typically, the involved muscles are the same as those affected during the original illness, but weakness may also occur in previously unaffected limb muscles. Progression of new symptoms is gradual, and affected persons are seldom severely disabled.[74,75] However, it may be dangerous in those with respiratory dysfunction or dysphagia. Population-based studies have suggested that the syndrome affects 20% to 85% of previously paralyzed patients.[72,75] The mean interval between acute poliomyelitis and the onset of postpoliomyelitis syndrome is 36 years. Although the cause is unknown, the leading theory is that late progression of muscle weakness is a result of physiologic attrition of motor units innervating muscles and muscle groups already less innervated as a result of earlier acute poliomyelitis.[72,75]

POLIOVIRUS IMMUNIZATION

Poliovirus Vaccines

Both IPV and live-attenuated OPV have been used effectively for more than 50 years to control polio. The introduction of Salk IPV in 1955 led to an immediate and dramatic reduction in both epidemic and endemic poliomyelitis in the United States and other Western countries. However, once the vaccine became generally available, it was observed that as many as 17% of children with paralytic poliomyelitis had received three or more doses of IPV, suggesting that the original formulation lacked optimum potency.[73] Meanwhile, live-attenuated OPV strains were developed by multiple passages of polioviruses in monkey kidney cell cultures and selection of mutants with low virulence for primates.[76] Successful field trials of the Sabin OPV vaccine strains were carried out in the United States and elsewhere from 1955 to 1959, and OPV was introduced for routine use in 1962 as separate monovalent vaccines; the trivalent product became available in 1964. OPV was quickly accepted by the pediatric and public health community because of superior immunogenicity compared with the original IPV formulation; lower cost; ease of administration; spread of vaccine virus to unimmunized, susceptible persons; and induction of gastrointestinal immunity. These properties have made OPV the logical choice for continued use in developing countries. However, because OPV also carries the risk of causing rare cases of VAPP, the United States and other developed countries have moved to exclusive use of a more potent IPV.[77]

Inactivated Poliovirus Vaccine

The majority of available IPV formulations worldwide are prepared by inactivation of wild-type, virulent poliovirus seed strains by the method originally developed by Jonas Salk (i.e., 1:1000 formalin treatment for 12–14 days at 37°C). All IPV preparations derived from WPVs currently available contain 40, 8, and 32 D-antigen units, respectively, for poliovirus serotypes 1, 2, and 3, formulated as either stand-alone trivalent IPV or together with other routinely administered childhood vaccines. In the United States, four doses of IPV are recommended at 2 months, 4 months, 6 to 18 months, and 4 to 6 years of age.[78]

Seroconversion rates are equal to, and mean antibody titers are superior to, those of OPV when given according to the same schedule. Studies conducted in the United States demonstrate that neutralizing antibodies are detectable to all three types in 99% of recipients after two doses and in 100% after the third dose.[79] A large boost in antibody titer follows the third dose.[79] After three doses, mean titers to types 1 and 3 are higher than in OPV-immunized children, whereas mean titers to type 2 are equivalent.

In tropical countries such as Cuba and in Puerto Rico, IPV administered at 3-dose schedules of 6, 10 and 14 weeks resulted in seroconversion in 94%, 83%, and 100% and 85.8%, 86.2%, and 96.9%, respectively, for poliovirus serotypes 1, 2, and 3, respectively.[80,81] In the latter country, a three-dose series similar to that used in the United States yielded seroconversion rates to poliovirus types, 1, 2, and 3 of 99.6%, 100.0%, and 99.1%.

Based on information derived from high-income countries, circulating antibody persists for decades and possibly for life. After a primary immunizing series of three or four doses, neutralizing antibodies are a usually found in all individuals at 5 years after immunization.[82] Although titers may decrease over time and fall below detectable levels, there is no evidence that this results in an increased susceptibility to paralytic poliomyelitis.

Several countries and the World Health Organization (WHO) have undertaken the development of IPV from the attenuated Sabin OPV strains.[83] Sabin-derived IPV (sIPV) is currently licensed for use in Japan and China. Possibly because of the vaccine strains used in creation and the reagents used to determine D-antigen concentration, the D-antigen content of each of the poliovirus serotypes varies among the various sIPV vaccines.[84,85] Furthermore, direct comparison of the D-antigen concentration of wild-type–derived IPV and sIPV is not possible. Protective antibodies against reference poliovirus strains, currently circulated wild poliovirus strains, and virtually all VDPV strains can be induced by sIPV.[83,86] The sole exception was a type 2 iVDPV that was not neutralized by sIPV or traditional IPV.[86] After administration

of a primary series vaccination in infants and a booster dose in adults, no differences in the decay of neutralizing antibodies were observed in those receiving sIPV when compared with individuals receiving traditional IPV.[87–89]

IPV-immunized children develop little or no measurable secretory antibody.[90,91] When challenged with live polioviruses, IPV-immunized children shed the challenge virus in their feces at a higher rate and a higher titer and for a longer period than OPV-immunized children, indicating a greater potential for asymptomatic infection and transmission of circulating polioviruses to unimmunized contacts.[91–93] Although this is widely considered to be a disadvantage of IPV, there is evidence that the universal use of IPV results in partial protection that extends to unvaccinated persons in the community, albeit less than the protection provided by OPV.[94,95]

Live-Attenuated Poliovirus Vaccine

OPV is no longer distributed in the United States.[77] Most trivalent OPV preparations made in other countries meet the WHO Expanded Program on Immunization minimum potency standards of $10^{6.0}$, $10^{5.0}$, and $10^{5.5}$ cell culture infectious dose ($CCID_{50}$) for poliovirus types 1, 2, and 3, respectively.[96]

Because the more efficient replication of type 2 OPV virus regularly interferes with the replication of types 1 and 3, a primary series of three doses is required to ensure seroconversion to all three serotypes.[97] Under normal conditions, infants respond to the first OPV with seroconversion rates of 50%, 85%, and 30% to serotypes 1, 2, and 3, respectively.[98] Two months after the second dose, more than 86% of infants have serum antibody to all three poliovirus serotypes, and 2 months after the third dose, the prevalence of antibody to all three types is more than 96%.[79,99] However, the excellent seroconversion rates observed in infants from industrialized nations are not seen in those of developing countries.[82,100] Reduced antibody response in these settings may be the result of a complex interaction among multiple factors including the vaccine and its delivery, the host, and their environment.

Detectable serum antibody to all three types persists in 84% to 98% of vaccinees 5 years after primary immunization,[101] and reexposure to vaccine viruses likely contributes to the maintenance of antibody levels in the population.[102,103] Secretory immunoglobulin A poliovirus antibody appears in oropharyngeal and duodenal secretions 1 to 3 weeks after OPV immunization and persists for at least 5 to 6 years.[90,104] Challenge studies have suggested that the intestinal immunity induced by OPV is similar to intestinal immunity after natural poliovirus infection.[105] The efficacy of OPV was never tested when WPVs circulated in the United States. However, data obtained during a type 1 poliovirus outbreak in Taiwan demonstrated estimated efficacy of 82%, 96%, and 98% for one, two, and three or more doses, respectively.[106]

Nonimmune OPV recipients shed vaccine viruses in the feces for 1 to 6 weeks and from the oropharynx for 1 to 3 weeks. The spread of OPV virus to unimmunized children is considered to be advantageous, especially in areas in which vaccine acceptance levels are low. The importance of this "backdoor" method of protecting the community is uncertain. A seroprevalence study in Houston and Detroit has found that 11% to 42% of 11- to 35-month-old children had antibody, despite receiving no prior OPV.[107]

Monovalent and Bivalent Oral Poliovirus Vaccines

The Sabin OPV was originally introduced in 1961 to 1962 as three individual monovalent vaccines that were administered separately. These were replaced in 1963 by the trivalent vaccine. The monovalent and bivalent types 1 and 3 OPV vaccines are more immunogenic than trivalent OPV for the corresponding serotype because of the absence of heterotypic interference from type 2 Sabin vaccine virus in the trivalent formulation. Monovalent and bivalent vaccines play major roles in the supplementary immunization activities (SIAs) of global poliovirus eradication effort in resource-limited settings.[108–111]

In 2014, the World Health Assembly recommended the worldwide withdrawal of the type 2 component from trivalent OPV.[112] This recommendation was based on the fact that indigenous wild poliovirus type 2 had not been detected since 1999, and up to 40% of VAPP cases are associated with the type 2 component in trivalent OPV. The recommendation took effect in April 2016 when all nations using trivalent OPV switched to bivalent OPV containing polio types 1 and 3. Successful global withdrawal of trivalent OPV was accomplished by mid-May 2016. Bivalent OPV has replaced trivalent OPV in all countries using OPV in their national immunization programs.[113]

Vaccine-Associated Paralytic Poliomyelitis

The only adverse reaction associated with OPV is the rare occurrence of VAPP. The WHO has estimated the incidence of VAPP to be 2 to 4 cases per 1,000,000 birth cohort per year in countries using OPV.[82] In the United States, it affected approximately 1 person per 2.6 million OPV doses distributed.[114] An average of 8 cases of VAPP were reported annually in the United States before OPV was removed from the immunization schedule in the late 1990s.[114] Estimates based on countries in which OPV was used recently place the risk of VAPP to be 3.8 cases per million births (range, 2.9–4.7).[115] In a review of the global burden of VAPP, the distribution of the Sabin strains most frequently associated with VAPP in recipient cases was as follows: type 3, 42%; type 2, 26%; type 1, 17%; and multiple serotypes, 15%.[115] Among contact cases of VAPP serotypes identified were type 3 in 37%, type 2 in 31%, type 1 in 20%, and multiple serotypes in 12%. Of recipient cases of VAPP, 71% occurred in patients younger than 1 year; 14%, 1 to 4 years old; 5%, 5 to 19 years old; and 10%, age 20 years or older. Approximately 75% of recipient VAPP cases are associated with the first dose of OPV. In developing countries, VAPP may result after the second or subsequent doses of OPV, with a concentration of cases in children 1 to 4 years old.[82] Among contact cases, 35% of patients were younger than 1 year; 33%, 1 to 5 years old; 8%, 5 to 19 years old; and 24%, age 20 years or older.[115] Fifty-two percent of contact VAPP patients had never been vaccinated. In the majority of recipient cases of VAPP, patients develop paralysis 7 to 21 days after the first feeding of OPV.[114] Contact VAPP cases (parents, family members, babysitters, or other household contacts) develop paralysis 20 to 29 days after the administration of OPV to a close contact. For immunocompetent patients, the clinical features and outcome of VAPP differ little from disease caused by WPVs.

Approximately 25% of reported VAPP cases occur in children and adults who are immunodeficient.[116] Most of these patients have transient or hereditary B-cell immunodeficiency, severe combined immunodeficiency syndrome, or common variable immunodeficiency. The risk of VAPP in newborn infants with a congenital B-cell immunodeficiency disorder is approximately 2000-fold higher than for immunocompetent infants. There is little evidence that immunodeficiency states that predominantly affect T-cell function increase the risk of VAPP. Similarly, there is little evidence that HIV infection, hematopoietic malignancy, or solid-organ transplantation increases the risk of VAPP.[117,118] Clinical features that distinguish VAPP in B-cell immunodeficient patients include an unusually long interval between the last OPV dose and onset of neurologic disease, with a typical range of 1 to 8 months, and as long as 12 years.[119,120] The illness is protracted and characterized by chronic meningitis; progressive neurologic dysfunction, suggesting involvement of both upper and lower motor neurons; and progression of paralysis over several weeks.[121,122] Immunodeficient patients have a much higher risk of dying from VAPP than immunocompetent patients. Although fewer than 20% of surviving VAPP patients excrete polioviruses for longer than 6 months,[119] fecal excretion of virus was estimated to occur for as long as 19 years in one immunodeficient patient.[123] Most VAPP cases in immunocompromised patients have been associated with type 2 OPV virus.[116]

The mechanism whereby the OPV viruses cause rare cases of paralytic disease is not completely understood. It is well known that OPV virus readily undergoes mutation during the brief period of intestinal replication and that isolates can be recovered that are neurovirulent for primates.[21] Most OPV recipients shed polioviruses that have reverted to the naturally occurring genotype in an analogous stem-loop structure in the 5′ noncoding region of the genome that affects initiation of transcription, which is strongly associated with attenuation for each of the three OPV serotypes.[22,124] Additional mutational events, mostly

within the protein coding region, probably contribute to reversion to neurovirulence.[22,124]

Use of Poliomyelitis Vaccines in the Developing World

Previously trivalent OPV and now bivalent OPV containing types 1 and 3 are used exclusively in developing nations for routine immunization because of lower cost, ease of administration, and superior secretory immunity in the gastrointestinal tract. In addition, OPV viruses are transmitted by immunized children to nonimmune contacts, a circumstance that is thought to be compounded by poor sanitation and crowded living conditions.

The WHO position paper on polio vaccines and polio immunization[83] recommends the inclusion of at least one dose of IPV for all countries using OPV, including all polio-endemic countries and countries at high risk for polio importation. The primary purpose of the IPV dose is to induce immunity to type 2 poliovirus, which could be rapidly boosted should an outbreak of type 2 disease occur after the global withdrawal of trivalent OPV. OPV should be administered according to the schedules of national immunization programs (e.g., at 6, 10, and 14 weeks or 2, 4, and 6 months). The dose of IPV can be given with the second or third dose of OPV. In addition, a dose of OPV should be administered as soon as possible after birth. Passively acquired maternal antibody present in the infant's circulation and in maternal colostrum blunts the immune response to the birth dose in some infants. However, infants who receive OPV at birth are more likely to have antibody to all three poliovirus types at 4 months of age.[125]

Unfortunately, many infants in tropical countries are left unprotected after receipt of the recommended number of OPV doses. Low seroconversion rates to three OPV doses have been widely documented,[125–130] averaging 73%, 90%, and 70% for types 1, 2, and 3, respectively.[100] This poor response appears to have contributed to poliomyelitis outbreaks in some countries with relatively high immunization rates.[130–132] Although the reasons for the lower potency of OPV in tropical areas remains incompletely understood, the effect of concurrent diarrheal illnesses has emerged as an important factor. Studies conducted in Brazil and Bangladesh have shown reduced seroconversion rates to types 2 and 3 OPV in infants with diarrhea at the time of OPV feeding, whereas the response to type 1 was not affected.[133,134] The impact of diarrhea on seroconversion persists despite the administration of three or four OPV doses. Similar effects of diarrheal disease have been observed on the immunogenicity of bivalent OPV in Nepalese children.[135] Malnourishment may be an additional factor influencing seroconversion. A study of Moroccan children found that seroprevalence rates for poliovirus types 1 to 3 were significantly lower among malnourished children.[136]

The low effectiveness of trivalent OPV in the presence of intercurrent diarrheal diseases has led to the introduction of other strategies to reduce poliovirus transmission in resource-poor settings. Monovalent and bivalent types 1 and 3 OPV vaccines have been introduced via supplemental immunization activities into many areas where WPVs continue to circulate. Studies in Egypt and Africa have confirmed the superior immunogenicity of monovalent OPV vaccines for newborn infants[108,111]; case-control studies in India and Nigeria have demonstrated that monovalent OPV type 1 vaccine is three to four times more effective per dose than trivalent OPV in protecting against paralytic poliomyelitis.[109,137] Comparisons of bivalent type 1 and 3 OPV vaccine to monovalent OPVs and trivalent OPV have demonstrated their noninferiority to the former and superiority to the latter,[110,138] resulting in WHO recommendations for their use in the global polio eradication program.[139] The effectiveness of monovalent and bivalent OPVs can be seen in India, where SIAs with these vaccines resulted in interruption of WPV circulation in January 2011.[1]

Historically, the costs associated with production and delivery and the requirements for injection have made the sole use of IPV an undesirable alternative for developing countries. WHO currently recommends the inclusion of at least one dose of IPV for all countries using OPV. In countries with a high vaccination coverage (90%–95%) and low importation risk, an IPV-OPV sequential schedule can be used. The purpose of this schedule is to provide protection against VAPP. For this schedule, WHO recommends one or two doses of IPV, followed by at least two doses of OPV.[82]

In general, IPV is highly immunogenic for infants in developing areas, although seroconversion and antibody titers are diminished, with short intervals between doses (e.g., 1 month), and also reduced in the presence of high titers of maternal antibody in young infants.[140–142] Studies have better defined the use of IPV in resource-poor areas of the world. In the 1980s, IPV was used as a supplement to OPV immunization in Israel, where type 1 poliovirus continued to cause epidemic disease in the Gaza Strip, despite relatively good rates of OPV coverage.[130,143] Israel switched exclusively to IPV in 2005, but in 2013 a dose of bivalent OPV was administered to all children born after 2005 to halt the circulation of a WPV detected in the sewage system.[144] IPV administered at 6 to 9 months of age has been shown to produce markedly enhanced seroconversion rates in infants given OPV at 2, 3, and 4 months of age in Côte d'Ivoire[145] and in Indian infants[146] for whom vaccination history could not be validated by clinic records. Last, a study conducted in Cuba documented that a single fractional dose of IPV administered intradermally can induce priming and seroconversion in more than 90% of immunized infants.[147]

Once the goal of polio eradication is achieved, the use of all live polio vaccines will cease in order to prevent outbreaks of VDPVs. IPVs will be required in order to maintain protection against reemergence of polio from immunodeficient individuals excreting virus, laboratory mishaps, or intentional reintroduction.

POLIOMYELITIS IN DEVELOPING NATIONS AND GLOBAL ERADICATION

Even after the introduction of polio vaccines, poliomyelitis was regarded as an epidemic disease of wealthier nations and was ignored in developing countries. However, surveys regarding lameness in schoolchildren in the 1960s and 1970s in more than 20 nations revealed lower limb paralysis prevalence rates of 2 to 11 per 1000 population, figures that reflect poliomyelitis incidence rates that equal or exceed those of the peak epidemic years in the United States.[148,149] Most cases of paralytic poliomyelitis in developing countries are caused by type 1 poliovirus and occur in children between the ages of 6 months and 2 years.

In 1974, WHO founded the Expanded Program on Immunization, which provided monetary and technical support for basic immunization against several childhood diseases, including polio, and created a worldwide standard polio immunization policy.[149] However, polio vaccines still failed to reach many children because of interrupted supplies, disruptions in the cold chain necessary to maintain the potency of OPV, civil strife, and poor political support. In 1983, an international conference held in Bellagio, Italy, soon after smallpox eradication, articulated the feasibility of worldwide poliomyelitis eradication,[150] and in 1988 the World Health Assembly set a goal of global eradication of poliomyelitis by the year 2000. Despite major progress, this goal has not yet been achieved.[1]

The WHO Global Poliomyelitis Eradication Initiative uses several major strategies to control and ultimately eradicate polio from most regions of the world, including encouragement of routine childhood immunization, SIAs, improvement of laboratory capabilities, intensified surveillance, and rapid response to identified outbreaks.[151] SIAs are highly coordinated nationwide or region-wide events in which all persons within a targeted age group receive two doses of OPV within a short interval.[152] Seroconversion rates during these mass campaigns are higher than for routine immunization, possibly because of the spread of OPV virus or because they are conducted during the dry season, when diarrheal disease is less prevalent.[153]

Progress toward eradication has been hampered by a number of unanticipated events, including a temporary but disastrous suspension of all immunization in northern Nigeria in 2003 to 2004 that led to importation of polio to 27 previously polio-free nations,[154] civil unrest in some locations leading to inadequate surveillance and vaccination activities,[155] and the emergence of cVDPV in many regions.[18,22] Renewed efforts and newly available resources have led to a dramatic reduction in global paralytic poliomyelitis cases to a low of 19 cases in 2017.[1]

There has been a complete disappearance of all cases caused by wild-type 2 poliovirus,[156] and the last known case of wild-type 3 poliovirus occurred in 2012.[1,156] In 2017, paralytic polio cases were reported from three nations where polio is considered to be endemic (Pakistan, Afghanistan, Nigeria).[1] In April 2013, WHO introduced the Polio Eradication and Endgame Strategic Plan 2013–2018, a 6-year plan for the global eradication of polio.[157,158] The four major objectives of the plan are outlined in Table 171.1.[157,158]

Vaccine-Derived Poliomyelitis Viruses

During 2000 and 2001, an outbreak of 21 cases of paralytic poliomyelitis on the island of Hispaniola occurred. This outbreak was caused by a virulent strain genetically related to the type 1 Sabin OPV vaccine strain.[26] Since then, at least 22 other outbreaks of paralytic disease caused by VDPVs have been discovered in underimmunized children living in economically deprived regions.[18,22] The low immunization rates in these areas have permitted these viruses to circulate for long periods and, by continuous mutation, acquire biologic properties indistinguishable from those of WPVs.[26]

The emergence of VDPV has profoundly influenced plans for the eventual cessation of poliovirus immunization after eradication of poliomyelitis, which will now include a strategy to discontinue OPV use worldwide, introduce IPV into as many countries as feasible, develop an OPV vaccine stockpile for use anywhere in the world, and develop a plan for containment of laboratory stocks of WPV and attenuated polioviruses.[22,157–159]

TABLE 171.1 World Health Organization (WHO) Polio Eradication and Endgame Strategic Plan 2013–2018

OBJECTIVE	PRIMARY GOAL
Poliovirus detection and interruption of its transmission	Stop all wild-type poliovirus transmission by the end of 2014
Strengthening of routine immunization programs and the withdrawal of OPV	Withdrawal of the type 2 component of OPV in all routine immunization programs by mid-2016 and use of bivalent OPV in 2019–2020
Containment and certification	Certification of all six WHO regions as having eradicated wild-type polioviruses by the end of 2018
Legacy planning	Have a strategy in place by the end of 2015 to ensure that the world remains permanently free of polio as well as to transition the lessons learned, with assets and infrastructure built to support this effort so that they may benefit other development goals and global health priorities

OPV, Oral polio vaccine.
From World Health Organization. Poliomyelitis: intensification of the Global Eradication Initiative. Report by the Secretariat. http://apps.who.int/gb/ebwha/pdf_files/WHA66/A66_18-en.pdf. Accessed July 31, 2018; and World Health Organization. Executive summary of the Polio Eradication and Endgame Strategic Plan 2013–2018. http://www.polioeradication.org/Portals/0/Document/Resources/StrategyWork/PEESP_ES_EN_A4.pdf. Accessed July 31, 2018.

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172 Coxsackieviruses, Echoviruses, and Numbered Enteroviruses (EV-A71, EVD-68, EVD-70)

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SHORT VIEW SUMMARY

Definition
- The coxsackieviruses, echoviruses, and numbered enteroviruses (EVs) are members of the genus *Enterovirus.*
- The four species, human EV-A to EV-D, within the genus *Enterovirus* total more than 100 serotypes.
- EVs are responsible for a wide array of clinical syndromes involving multiple organ systems, including acute meningitis, encephalitis, paralysis, exanthems, hand-foot-and-mouth disease, herpangina, myopericarditis, pleurodynia, and acute hemorrhagic conjunctivitis.

Epidemiology
- The EVs are found worldwide.
- In regions with temperate climates the majority of EV infections occur during the summer and early autumn months. In tropical regions infections occur year-round, with increased frequency during the rainy season.
- A large outbreak of EV-D68 occurred in the United States in 2014.

Microbiology
- Morphologically, they are small (30 nm in diameter), nonenveloped, icosahedral-shaped viruses that possess a single-stranded, positive-sense RNA genome.

Diagnosis
- EVs can be isolated, or their genomic RNA detected, from throat secretions, feces, cerebrospinal fluid, blood, and various tissues (heart, brain) and fluids (urine, pericardial, vesicle).
- Nucleic acid amplification testing has been shown to be quicker and more sensitive than cell culture for EV detection.
- Serotype identification is accomplished by sequencing of the major capsid protein VP1.

Therapy
- Therapy is supportive.
- No specific antiviral therapy is currently available for the treatment of EV infections.

Prevention
- Contagion can be prevented by hand washing and, in the case of certain serotypes, avoidance of EV-contaminated fomites.
- Vaccines for the prevention of human EV-A71 infection are available in Asia.

This chapter covers human disease caused by the group A coxsackieviruses (CVs), group B CVs, echoviruses (Es), and the numbered enteroviruses (EVs), which are distributed among four species, EV-A to EV-D, of the genus *Enterovirus.* Viral diseases caused by the closely related and newly designated genus *Parechovirus*, of the Picornaviridae, are discussed in Chapter 173. These viruses have many physical, epidemiologic, and pathogenetic characteristics in common, as described in Chapter 170. Greater than 90% of infections caused by the nonpolio EVs are asymptomatic or result only in undifferentiated febrile illness.[1] When disease occurs, the spectrum and severity of clinical manifestations vary with the age, gender, and immune status of the host and with the subgroup, serotype, and even the intratypic EV strain.

Some clinical syndromes (viral meningitis and some exanthems) are caused by many EV serotypes, some are predominately caused by certain EV serotypes (e.g., pleurodynia and myocarditis by the group B CVs), and other diseases are mostly associated with individual EV serotypes. Infections caused by some of the more recently recognized serotypes are discussed at the end of this chapter.

CENTRAL NERVOUS SYSTEM INFECTIONS

Acute Viral Meningitis

Viral infection is the dominant cause of the aseptic meningitis syndrome, and the EV-B species, which encompass all of the group B coxsackievirus (CV-B) and echovirus (E) serotypes, cause most acute viral meningitis cases in both adults and children.[2,3] Group A coxsackieviruses (CV-A) cause relatively fewer cases.[2] Historically, CV-B2 to CV-B5 and E-4, -6, -7, -9, -11, -13, -16, -18, -30, and -33 are the most frequently implicated serotypes. On occasion a single E serotype may cause widespread outbreaks; for example, E-13 caused outbreaks of aseptic meningitis throughout Europe and the United States in 2000 and 2001,[4] and E-33 caused widespread disease in New Zealand during the winter of 2000.[5]

Clinical Manifestations

Infants younger than 3 months have the highest rates of clinically recognized aseptic meningitis, in part because lumbar punctures are routinely performed for evaluation of fever in this age group.[6] Only a minority of these infants have clinical manifestations suggestive of neurologic disease.[7]

The severity of disease in older children and adults with aseptic meningitis varies widely. The onset may be gradual or abrupt, and the typical patient has a brief prodrome of fever and chills. Headache is usually a prominent complaint. Meningismus, when present, varies from mild to severe. Kernig and Brudzinski signs are present in only about one-third of patients. Signs of meningeal irritation are less frequently observed in young infants than adults.[6,8] Pharyngitis and other symptoms of upper respiratory tract infections are often present. The illness is sometimes biphasic, as in poliomyelitis; these patients present with a prodromal illness with fever and myalgias, followed by defervescence and absence of symptoms for a few days, and then experience abrupt recurrence of fever with headache and other signs of meningismus. Complications such as febrile seizures, complex seizures, lethargy, coma, movement disorders, and development of a syndrome of inappropriate antidiuretic hormone secretion occur early in the course of aseptic meningitis in 5% to 10% of patients.[6,9,10] Adults may experience a more prolonged period of fever and headache than infants and children, and some adult patients may take weeks to return to normal activity.[8,11]

Laboratory Diagnosis

The clinical diagnosis of viral meningitis depends on routine examination of cerebrospinal fluid (CSF). The CSF is clear and under normal or mildly increased pressure. The total CSF cell count is usually 10 to 500/mm^3 but may occasionally exceed 1000/mm^3. Cell counts less than 10 cells/mm^3 may occur.[2,12,13] Absence of pleocytosis may occur in 18% to 30% of infants and children with EV meningitis detected by nucleic acid amplification testing (NAAT) and occurs more frequently in neonates