cardiovascular disease.[75] Another study of liver transplant recipients with ciHHV-6 showed a higher rate of bacterial infections.[76] Grade 2 to 4 acute graft-versus-host disease was more common when HSCT donors or recipients had ciHHV-6, although there was no difference in chronic graft-versus-host disease, engraftment, or mortality.[77]

### Therapy

HHV-6 is sensitive to ganciclovir, foscarnet, and cidofovir in vitro; the latter two agents are more active in cell culture.[78] Brincidofovir, the lipophilic prodrug of cidofovir that can be given orally, has also been shown to be active against HHV-6A and HHV-6B in cell culture.[79] HHV-6, like CMV, is not sensitive to acyclovir. HHV-6 U69 is a protein kinase that phosphorylates ganciclovir. HHV-6 DNA levels in the CSF and serum declined with ganciclovir or foscarnet therapy in one series; however, without a control group it is unknown whether this was treatment related.[68] Antiviral therapy has been used in some immunocompromised patients, but no controlled studies have shown that these drugs are effective. Some anecdotal reports have suggested that patients with HHV-6 encephalitis may have responded to a 7-day course of ganciclovir or foscarnet. Either drug, or the combination of both drugs, is recommended for immunocompromised patients with HHV-6 encephalitis.[80] Patients receiving full-dose therapy (foscarnet ≥180 mg/kg or ganciclovir ≥10 mg/kg) had a better response than those receiving lower doses; the difference in responses between the two drugs was not significant.[51] Ganciclovir-resistant HHV-6, due to a mutation in the viral U69 protein kinase, has been isolated from a patient with AIDS.[81] Prophylaxis with ganciclovir or foscarnet has been reported to reduce HHV-6 reactivation or encephalitis in small studies,[82–85] but this is not recommended owing to the toxicity of the drugs and the low incidence of disease in immunocompromised patients. Intensive prophylaxis (ganciclovir 5 mg/kg daily on days −8 to −2 during conditioning followed by valacyclovir 2 g every 8 hours for the first 100 days after transplant) reduced HHV-6 reactivation in a small prospective study of cord blood transplant recipients.[60]

A number of small molecules that inhibit HHV-6 are being investigated.[78] These include cyclopropavir[86] and benzimidazole analogues,[87] which have been shown to have activity against HHV-6. HHV-6–specific T-cell immunotherapy has been shown to reduce disease in HSCT recipients with HHV-6 reactivation.[88,89] Work on development of antivirals and on adaptive immunotherapy for HHV-6 and HHV-7 infections has been reviewed as part of the summary of the 9th International Conference on Human Herpesviruses 6 and 7.[90]

## HUMAN HERPESVIRUS TYPE 7

### History

HHV-7 was discovered by Frenkel and colleagues[91] in 1990 in a healthy person and was shown to be a cause of exanthem subitum.[92]

### Description of the Virus

HHV-7, like HHV-6, is a member of the *Roseolovirus* genus and shares 20% to 75% amino-acid identity with HHV-6 in many of their viral proteins.[5] The HHV-7 genome contains about 145 kilobase pairs of DNA.

### Epidemiology

HHV-7 infections occur at a later age than HHV-6 infections (see Fig. 139.1).[7] About 18% of children are infected with HHV-7 by 1 year of age and 53% by 2 years. Most children are infected between ages 2 and 5, presumably from infected saliva of parents and siblings.[93] HHV-7 DNA was detected in PBMCs from 67%, and in cervical swabs from 3%, of pregnant women.[8] About 50% of HSCT and 20% of solid-organ transplant recipients reactivate HHV-7 as indicated by viral DNA in the peripheral blood.[14,94]

### Pathogenesis

HHV-7 has a narrower tissue tropism than HHV-6. HHV-7 infects $CD4^+$ T cells, epithelial cells in the salivary glands, and cells in the lungs and skin. HHV-7 is frequently shed in saliva at high levels throughout life in most adults and children.[95] The virus has been detected in breast milk and establishes latency in $CD4^+$ cells. HHV-7 induces degradation of MHC class I molecules.

### Clinical Manifestations

Primary HHV-7 infection may be asymptomatic or associated with fever or febrile seizures. In a study of 30 children with HHV-7 viremia, the most common clinical presentation was seizures, which occurred at 12 to 63 months of age; 10 of 12 patients had febrile seizures.[7] HHV-7 viremia was present in 7% of children with febrile status epilepticus; 5% of patients had primary HHV-6B infection and 2% had virus reactivation.[22]

The second most common presentation of HHV-7 viremia was nonspecific fever with a mean temperature of 40.1°C. Less common symptoms were upper respiratory tract disease, vomiting, and diarrhea. Leukopenia was frequently noted. In a study of 496 children presenting to the emergency department, children with HHV-7 had a similar level of fever, rash, and gastrointestinal symptoms but were older and more likely to have seizures than those with HHV-6.[96]

HHV-7 is also a cause of exanthem subitum, although most cases are due to HHV-6. HHV-7 has been less frequently associated with CNS disease than HHV-6, but two cases of hemiplegia associated with HHV-7 have been described. HHV-7 has been associated with encephalitis in immunocompetent[97,98] and immunosuppressed[99] patients. Ten percent (15/156) of young children hospitalized in Britain and Ireland with encephalitis or fever and seizures were found to have an acute HHV-7 infection.[64] HHV-7 was not reported to cause congenital infection (defined as viral DNA in cord blood) in more than 5600 births.[40]

### Laboratory Diagnosis

Like HHV-6, the most common diagnostic test for HHV-7 in children is seroconversion based on detection of antibody by indirect immunofluorescence assay or ELISA.[64] Detection of HHV-7 in serum or plasma is much less common than for HHV-6; therefore the presence of HHV-7 DNA in blood in the absence of antibody is more likely to be indicative of acute infection. HHV-7 has been cultured from PBMCs of patients with exanthem subitum, but this is done only in research laboratories.

Unlike HHV-6, levels of HHV-7 in the blood did not correlate with disease in immunocompromised patients.[13] Because HHV-7 DNA has been detected in the brain by PCR in 37% of adults,[100] detection of HHV-7 protein is more specific than viral DNA for the diagnosis of encephalitis.

### Therapy

Like HHV-6, HHV-7 is most susceptible to foscarnet and cidofovir in vitro, although virus replication is also inhibited by ganciclovir. There are insufficient clinical reports to indicate whether these drugs are effective in vivo.

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*The complete reference list is available online at Expert Consult.*

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# 140 Kaposi Sarcoma–Associated Herpesvirus (Human Herpesvirus 8)

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**SHORT VIEW SUMMARY**

**Definition**
- Kaposi sarcoma–associated herpesvirus (KSHV), or human herpesvirus 8 (HHV-8), is the etiologic agent of Kaposi sarcoma and primary effusion lymphoma and is tightly linked with multicentric Castleman disease.

**Virology and Epidemiology**
- KSHV establishes lifelong infection, primarily persisting in latently infected B lymphocytes.
- Replication occurs in oral epithelium, and infectious KSHV is present in the saliva of asymptomatic seropositive individuals.
- Transmission is predominantly the result of exposure to infected saliva.
- Primary infection is usually asymptomatic and rarely recognized.
- In contrast to other herpesviruses, seroprevalence of KSHV varies significantly throughout the world and is highest in sub-Saharan Africa and the Mediterranean region and in men who have sex with men in the United States.
- KSHV malignancy usually occurs in the setting of immune suppression.

**Microbiology**
- KSHV is a gamma-2 herpesvirus, genus *Rhadinovirus*.
- KSHV is an enveloped, double-stranded DNA virus.
- KSHV is also known as human herpesvirus 8 (HHV-8).

**Diagnosis**
- KS can be diagnosed by its clinical appearance and confirmed by biopsy.
- Primary effusion lymphoma and multicentric Castleman disease are diagnosed by biopsy.
- KSHV infection can be diagnosed serologically, although assays are not standardized.

**Therapy**
- Antiviral therapy is currently only available for the lytic stage of infection, and is of no proven benefit in the treatment of KSHV malignancies.
- Enhancing immunity with antiretroviral therapy in HIV infection, or through reduction of immune suppression in transplantation, can lead to KS regression.
- Cytotoxic approaches are often necessary for KSHV malignancy.

**Prevention**
- There is currently no KSHV vaccine.

Kaposi sarcoma (KS)–associated herpesvirus (KSHV), or human herpesvirus 8 (HHV-8), is the eighth and most recently discovered human herpesvirus. KSHV was discovered as a result of its connection with KS and is also linked with primary effusion lymphoma (PEL) and multicentric Castleman disease. The role of KSHV in malignancy has generated much interest in this virus.

## HISTORY

Kaposi sarcoma was first described in 1872 by Moritz Kaposi, a prominent Hungarian dermatologist.[1] Kaposi described findings in five men of "idiopathic multiple pigmented sarcoma of the skin."[2] He noted aggressive disease and emphasized that the syndrome was incurable and rapidly lethal.[3] In fact, three of the men reported by Kaposi were dead within 16 months of presentation, and autopsy demonstrated disseminated disease. Despite the aggressive nature of the disease Kaposi described, KS subsequently came to be regarded as an indolent disease in elderly men of Mediterranean and eastern European descent. It is not clear what accounted for the evolution in the defining features of KS from the aggressive, rapidly fatal disorder described by Kaposi to a relatively mild one. During the 1950s, KS was recognized as an important disease in areas of sub-Saharan Africa.[4] Then, in 1981, Alvin Friedman-Kein reported on 50 young men who had had sex with men with KS of the skin, lymph nodes, mucosa, and viscera.[5] This report heralded the acquired immunodeficiency syndrome (AIDS) epidemic. The similarity of the original syndrome described by Kaposi and that seen in human immunodeficiency virus (HIV) infection is striking and raises the question of whether AIDS-like immune suppression was present in the men initially described.[3]

### Discovery of KSHV

KSHV was identified in 1994 by Chang and Moore and coworkers[6] in KS lesions by using a polymerase chain reaction (PCR)–based technique termed *representational difference analysis*. This technique searches for DNA, such as from a virus, that is present in diseased tissue and absent in normal tissue.[7] These investigations were based on epidemiologic observations suggesting that an infectious agent may have an etiologic role in KS. KS occurred at a 20-fold higher rate in men who had had sex with men who had AIDS, compared with those who contracted AIDS by other means, such as by a bloodborne route. Subsequent to this seminal discovery, work by many groups worldwide has elucidated much about this virus.

## CLASSIFICATION AND BIOLOGY

KSHV is the only known human rhadinovirus (gamma-2 herpesvirus) and is related to other rhadinoviruses, including those that infect New World (South American) and Old World (African) monkeys and rodents (murine gammaherpesvirus 68).[8–12] Two Old World monkey rhadinoviruses (RFHVMm and RFHVMn) are found in retroperitoneal fibromatosis in monkeys. This entity has histologic similarities to KS. *Herpesvirus saimiri* (HVS), a New World virus, can cause T-cell lymphoma when infecting New World monkeys that are not its natural host.[13] Epstein-Barr virus, a gamma-1 herpesvirus, is KSHV's closest human relative.

### Virus Description

KSHV is an enveloped virus that measures 140 nm in diameter, and its appearance by electron microscopy is indistinguishable from that of other herpesviruses.[14,15] The KSHV genome contains approximately 140 kb of unique sequence,[8,16] which encodes approximately 100 open reading frames (ORFs). The nomenclature of the ORFs is based on that of HVS because of high sequence and positional homology with those of HVS and the fact that HVS was the only fully sequenced gamma-2 herpesvirus before KSHV. ORFs without homology to those in HVS are numbered sequentially with K prefixes. A number of KSHV genes are homologues of human genes that were presumably "pirated" from mammalian cells during the evolution of the virus. The unique KSHV

sequence is flanked by approximately 40 copies[17] of 0.8-kb guanine- and cytosine-rich terminal repeat elements. This translates to the terminal elements comprising about 20% of the viral genetic sequence, a feature common to the gamma-2 herpesviruses. This large devotion of energy to the terminal repeats is likely due to their central role in KSHV persistence during latent infection.

## KSHV Entry Into Cells

KSHV attaches to cells before entry by binding to cell surface heparin sulfate, integrins (including $\alpha_3\beta_1$), and cysteine transporter xCT.[18,19] The bound virus is then translocated to lipid rafts and binds to the EphA2 receptor, where internalization occurs.[20,21] Binding of these cell receptors activates signaling cascades that facilitate virus entry into the cell by endocytosis or macropinocytosis, and the virus enters the cell in endosomes. The virus envelope then fuses with the endosomal membrane to release the virus capsid, which traffics to the nuclear pore, where it delivers virus DNA into the nucleus.[1]

## Lytic Virus Infection

KSHV is capable of both latent and lytic infection.[9,22,23] During lytic infection, many encapsidated viral progeny are produced in a cell and then released as the infected cell dies. Almost all of the nearly 100 KSHV genes are devoted to, and only expressed during, lytic infection. These genes encode proteins responsible for replication of the viral DNA and packaging the DNA into capsids. The viral genome is linear, with terminal repeats on each end when packaged in viral capsids. In addition to genes involved in virus replication, some genes expressed during lytic infection are involved in immune evasion, preventing the host from properly responding to and targeting the infected cells.[24–26]

## Latent Virus Infection

Latent KSHV infection sharply contrasts with lytic infection.[22,23,27,28] Latent infection predominates over lytic infection in KSHV-infected tumors and cell lines, with only a small fraction of infected cells undergoing lytic infection. The primary reservoir for KSHV is likely circulating B lymphocytes. Because of its capacity for latent infection, KSHV persistence in its human host is lifelong, similar to other herpesviruses. In latently infected cells, the viral genome circularizes by fusing at its terminal repeat ends and persists as a multiple-copy (ranging in number from 10 to 50 copies) extrachromosomal episome (plasmid) within the nucleus. Only approximately five KSHV genes are expressed during latent infection. Rather than causing cell death, these genes encourage cell survival. Because promotion of cell survival is also a prominent feature of malignancy, it is not surprising that KSHV is associated with certain tumors.[29]

## KSHV Gene Expression in Latent Infection

Genes expressed in latent infection have important roles in tumorigenesis.[30–34] To persist in latent infection in proliferating cells such as tumor cells, KSHV episomes must replicate and efficiently segregate to progeny nuclei. The viral latency-associated nuclear antigen (LANA, or ORF73) acts on a specific sequence in the virus terminal repeat DNA to mediate KSHV DNA replication and to tether episomes to chromosomes during mitosis to ensure efficient segregation to daughter cells. LANA also exerts effects on transcriptional regulation and cell growth. Viral cyclin D (ORF72) is a homologue of cell cyclin D and stimulates the $G_1$-to-S transition of the cell cycle. The viral cyclin D is resistant to the multiple inhibitors that normally inhibit cell cyclin D, resulting in unchecked cell growth. The KSHV viral FADD-like interleukin-1β–converting enzyme (FLICE)–inhibitory protein (vFLIP, or K13) activates nuclear factor kappa B (NF-κB) and inhibits apoptosis, thereby preventing the cell from eliminating itself once it is infected. Notably, LANA, the viral cyclin, and vFLIP are consistently expressed in all latently infected cells from a single promoter. The kaposin locus encodes overlapping ORFs, and this transcript and its protein products are induced in lytic infection. Kaposin A (K12) has been reported to exert transforming effects, and kaposin B acts to increase the expression of cytokines. Perhaps the most important function of the kaposin transcript is the expression of viral microRNAs. These microRNAs include an orthologue of miR-155, which affects B-cell differentiation, and other microRNAs that target inhibitors of NF-κB and of a cell cyclin–dependent kinase, thereby promoting NF-κB activity and cell cycle progression. Latency-associated membrane protein (LAMP, or K15) interacts with growth control proteins. LANA2 (vIRF3) is expressed in B cells, not in KS tissue, and inhibits apoptosis.

## Possible Paracrine Effects of Lytic Virus Infection

Although only a small percentage (≈1%) of cells within tumors undergoes lytic infection, these cells may also have an important role in tumorigenesis. For instance, in lytic infection, a G protein–coupled receptor homologue (ORF74) that is constitutively active and has paracrine effects is expressed.[35] Therefore, although the cell with lytic infection will die, it can produce factors that have growth effects on nearby cells. In fact, transgenic mice expressing this viral protein have KS-like lesions.[36,37]

## Laboratory Infection Models

Cell culture and transformation models for KSHV remain limited. Primary bone marrow endothelial cells can be infected and transformed, but only approximately 5% of the cells are infected, with paracrine effects stimulating growth in the other cells.[38] Primary rat mesenchymal precursor cells are efficiently transformed by KSHV and can serve as a useful tool to assess KSHV transforming function, although harvesting these cells requires specialized expertise.[39] Because of a lack of a cell line permissive for KSHV lytic replication, the mainstay of KSHV production is from cell lines derived from KSHV primary effusion lymphomas. The vast majority of cells in these lines are latently infected, but lytic infection can be induced by several methods, such as incubation with phorbol esters, to produce infectious virus, albeit at relatively low titers. The most tractable models so far used to study the effects of KSHV virus infection are in dermal microvascular cells.[23] KSHV induces phenotypic changes in these cells, such as spindle formation, but does not immortalize or fully transform them.

# PATHOGENESIS

## Suppression of Immunity as a Factor Leading to KSHV Malignancy

KSHV has an etiologic role in KS, PEL, and multicentric Castleman disease. Overall, KSHV is well adapted to its human host and usually does not cause disease. Such a situation is ideal from the point of view of the virus because a commensal existence without harm to its host enhances its long-term survival. Suppression of the immune system appears to disturb the delicate balance between KSHV and its human host and can lead to KSHV-associated malignancy. However, other poorly understood factors also contribute to tumorigenesis. For instance, the cause of the more frequent occurrence of KS in men rather than in women, despite a similar prevalence of KSHV infection in many instances, is not clear. Furthermore, before the HIV epidemic, KS occurred relatively frequently in Uganda and Cameroon but not in Botswana and the Gambia, despite KSHV infection being common to all these countries.[40] These findings argue for as yet unknown factors interacting with KSHV to induce KS.

## KSHV and Inflammation

There is an interesting link between inflammation and KSHV pathogenesis despite the fact the depression of immunity is the typical scenario leading to KSHV malignancy. Inflammatory cell infiltrates composed of lymphocytes, plasma cells, and macrophages are often found in KS. In addition, KS can occur at sites of trauma (Koebner phenomenon). Most notably, the immune reconstitution inflammatory syndrome (IRIS) (see "KS-Associated IRIS After Institution of Antiretroviral Therapy" later), in the setting of antiretroviral therapy (ART) for HIV infection, can exacerbate or lead to KS. Cultured endothelial cells infected with KSHV express a number of inflammatory cytokines and chemokines, including interleukin (IL)-6.[2] Cyclooxgenase-2 (COX-2) is expressed in KS and in infected cultured cells and leads to chemokine secretion.[3] Notably, KSHV expresses a viral IL-6 homologue (vIL6) and also viral chemokines. vIL6 and IL-6 are both significantly elevated and believed to be an important component of multicentric Castleman disease (see

under "Clinical Manifestations" later).[4,5] KSHV-encoded chemokines and vIL6 are expressed during lytic virus infection, although vIL6 can also be expressed in latency. Despite the clear linkage between KSHV and inflammation, much remains to be elucidated regarding the role of inflammation in KSHV pathogenesis.

## EPIDEMIOLOGY

### Assays to Identify KSHV Infection

Assays to identify KSHV-infected individuals are still evolving.[9,23,41–43] Serologic assays for antibodies against specific KSHV antigens expressed during the latent or lytic phases of infection have been most commonly used. The assays differ in sensitivity and specificity, resulting in some that likely overestimate and others that underestimate seropositivity. With these limitations in mind, certain general conclusions regarding prevalence of KSHV infection can be made. Detection of KSHV DNA by PCR assay of blood is less sensitive than the serologic assays, reflecting highly variable levels of viremia occurring in both those with and those without KSHV-induced disease.

### Geographic Variance of KSHV Seroprevalence in Contrast to Other Herpesviruses

KSHV differs from other herpesviruses in that it does not cause worldwide ubiquitous infection.[9,23,41] Instead, the prevalence of infection in the general population varies significantly in different areas of the world. Sub-Saharan Africa has the highest rate of infection, with approximately 50% of the population infected. Seroprevalence is approximately 10% in the Mediterranean region, although in certain areas of Italy it approaches 30%. Seroprevalence in the United States and northern Europe is approximately 5%, but only 0.2% of individuals in Japan are positive. Despite the low prevalence of KSHV in the general population in the United States, approximately 15% to 20% of HIV-negative and approximately 40% of HIV-positive men who have sex with men are KSHV seropositive.[44] In contrast to the general population, approximately 90% to 100% of individuals with KS are seropositive, consistent with KSHV's etiologic role in this disease.

### KSHV Transmission

There are several patterns of KSHV transmission. In the United States, KSHV is spread predominantly through sexual contact among men who have sex with men. Among men who have sex with men, KSHV seropositivity is associated with high numbers of sexual partners, a history of sexually transmitted diseases, and the use of amyl nitrates.[44,45] In contrast to the well-documented sexual transmission among men who have sex with men, the evidence for heterosexual KSHV transmission is conflicting.[46–50] In areas of the world where KSHV infection is more prevalent, nonsexual transmission also occurs, and KSHV infection occurs among children before they are sexually active.[51,52] Intrafamilial clustering has also been documented as further evidence of nonsexual transmission.[53] Saliva is likely a unifying vehicle of both sexual and nonsexual KSHV transmission. Relatively high titers of KSHV DNA can be found in the saliva of infected individuals, likely produced from lytic infection in oral epithelial cells, whereas high levels of virus are not found at other sites. In one study of 50 KSHV-infected men who had sex with men without KS, 30% of oropharyngeal samples, compared with 1% of anal and genital samples, were positive for KSHV. KSHV from the oral cavity was 2.5 logarithms higher than the titer at other sites.[44] Interestingly, deep ("French") kissing was a risk factor for KSHV transmission among men who have sex with men.[44] Human leukocyte antigen alleles may influence the degree of KSHV shedding in saliva.[54] Solid-organ transplantation from a seropositive donor to a seronegative recipient has also been shown to transmit KSHV.[55] Vertical transmission from mother to infant can occur but appears to be rare.[56] Transmission by blood transfusion can also occur, and the risk is greatest in regions of high KSHV seroprevalence. Whether or not the blood supply should be screened in the United States, where the seroprevalence is low, remains controversial. However, there is currently no US Food and Drug Administration–approved diagnostic test for KSHV infection, limiting potential strategies to screen blood products for KSHV infection.[57–59]

## CLINICAL MANIFESTATIONS

### Primary Infection

A primary infection syndrome for KSHV has not been clearly described, and most infections are probably asymptomatic or unrecognized. In a prospective Egyptian study, 86 children ages 1 to 4 years presenting to the emergency department with fever of unclear origin were evaluated for KSHV infection. Six of the children likely had primary KSHV infection because they were seronegative but had KSHV DNA detected in saliva. In three of these subjects, follow-up serology was obtained, and all three seroconverted to KSHV. All but one of the six had a maculopapular rash that began on the face and gradually spread downward over the trunk and extremities. Five of the six also had associated upper respiratory tract symptoms. Fever persisted for a median of 10 days.[60] Primary KSHV infection was associated with mild symptoms of diarrhea, fatigue, localized rash (ankle and face), and lymphadenopathy (cervical and submental) in four of five HIV-negative men.[61] A 43-year-old HIV-infected man developed fever, arthralgia, cervical lymphadenopathy, and splenomegaly 5 weeks after KSHV seroconversion, and his illness spontaneously resolved within 10 weeks. Biopsies showed angiolymphoid hyperplasia and foci of KS. Neither lesions nor clinical symptoms had recurred after 8 years of follow-up (on ART).[62] Four months after transplantation, two renal allograft recipients developed primary KSHV infection from the same KSHV-positive donor. One recipient developed disseminated KS and the other a syndrome of fever, splenomegaly, cytopenia, and marrow failure with plasmacytosis. KSHV infection of immature progenitor cells from the aplastic bone marrow was noted for the patient with marrow failure.[63] Although these data are very limited, it appears that primary infection in immunocompetent hosts is self-limited, whereas primary infection in immunosuppressed hosts can be severe and have significant consequences.

### Kaposi Sarcoma

KS typically involves the skin and manifests as lesions that enlarge from patches to plaques to nodules.[64,65] The lesions often begin as violaceous and later evolve into a brown color because of hemosiderin deposition (Fig. 140.1). KS lesions are composed of vascular spaces, extravasated erythrocytes, and several different types of cells (Fig. 140.2). These include the malignant spindle cells and infiltrating mononuclear cells, such as hemosiderin-laden macrophages. The highly vascular nature of KS gives it its purple color. In the nodular stage, nearly all spindle cells are KSHV infected (see Fig. 140.2). KS is typically not monoclonal, and different tumor nodules in individuals may have different origins.[2]

### Four Epidemiologic Forms of KS

There are four variants of KS: classic, endemic, epidemic, and iatrogenic, which differ epidemiologically and clinically.[9,23,66,67] The occurrence of KS largely reflects the seroprevalence of the population, with KS more common in areas with high KSHV seropositivity. *Classic KS* occurs in elderly men of Mediterranean or eastern European descent, predominantly involves the skin of the lower extremities, and is indolent. *Endemic KS* occurs in certain sub-Saharan African countries. At least two forms of endemic KS occurred before the HIV epidemic. In adults, cutaneous KS occurred in an approximately 20:1 ratio of men to women; it clinically resembled classic KS in adults. However, in children younger than 10 years, KS caused an aggressive, multifocal, lymphadenopathic form, often without cutaneous lesions, that was frequently fatal.[64,65,68]

*Epidemic KS,* which refers to KS in HIV-infected individuals, tends to be aggressive, commonly involving the skin, gastrointestinal tract, and respiratory tract. KS in the lung often has lesions present in the bronchial mucosa but may be associated with a variety of radiographic manifestations, including nodules, adenopathy, and pleural effusions. In contrast to the lesions of classic KS, lesions in epidemic KS commonly involve the face (often the nose), genitalia, and oral cavity (palatal and gingival), in addition to the lower extremities.[69] This form is most common in the United States, where it predominantly affects men who have sex with men. However, KS largely occurs in heterosexual HIV-infected individuals in Africa. Since the start of the HIV epidemic in Africa, the ratio of men to women with KS has dropped 10-fold to


**FIG. 140.1 Kaposi sarcoma (KS) of the foot (A) and leg (B) in two human immunodeficiency virus–positive patients.** Lesions are highly vascular and often occur on the lower extremities. Newer KS lesions are typically violaceous (A) and evolve to a brownish color (B) over time because of hemosiderin deposition. (Numbered labels in A are present as part of a clinical treatment trial.) *(Courtesy Bruce Dezube, MD.)*

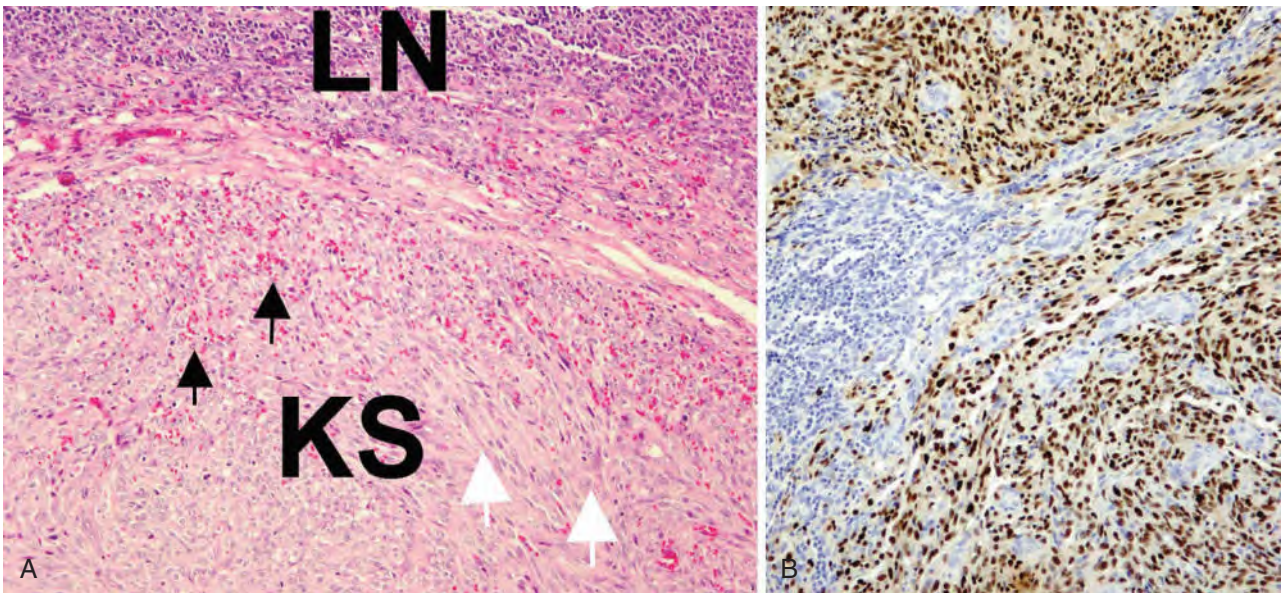


**FIG. 140.2 Kaposi sarcoma *(KS)* involving a lymph node.** (A) A spindle cell proliferation *(white arrows)* containing poorly formed vascular spaces with entrapped red blood cells *(black arrows).* Areas of uninvolved lymph node *(LN)* are seen at the top. (Hematoxylin and eosin stain.) (B) Immunohistochemical detection of Kaposi sarcoma–associated herpesvirus (KSHV) latency-associated nuclear antigen *(brown)* in the nuclei of many spindle cells indicates KSHV infection (×200). *(Courtesy Dan Jones, MD, PhD.)*

approximately 2:1. The number of childhood cases of KS has also significantly increased in Africa with the AIDS epidemic.[70,71] For instance, in Zambia in the early 1980s, KS accounted for 0% to 2% of childhood malignancies, but by 1992 it accounted for approximately 25% of childhood malignancies.[71–73] *Iatrogenic KS* occurs in individuals who are immunosuppressed, such as from organ transplantation, and tends to be aggressive. Kidney allograft recipients appear to be at higher risk for developing KS compared with other transplant recipients.[74]

The incidence of KS in HIV infection has decreased significantly in developed countries since the introduction of ART, but the standardized incidence rate remains very high for KS compared with other cancers in HIV infection.[75,76]

## Diagnosis

Although KS can often be recognized by a trained observer, the diagnosis is easily confirmed by biopsy.[65,69] Early stages of KS can be more difficult to recognize. The differential diagnosis of KS includes bacillary angiomatosis, which is caused by *Bartonella* species. Skin lesions of bacillary angiomatosis are very vascular and may mimic those of KS.[65]

## KSHV Viral Load Measurement

The measurement of KSHV viral loads has been performed on peripheral blood of patients with KS. Viral loads are performed by PCR assay of viral DNA either in plasma or in peripheral blood mononuclear cells (PBMCs), and studies vary as to which blood component is used. One study showed that detection of KSHV DNA in PBMCs of HIV-infected individuals without KS predicted the development of KS lesions.[77] Plasma KSHV DNA levels were greater in more advanced KS disease compared with less advanced disease; they were also greater in AIDS KS compared with classic KS.[78,79] KSHV levels were higher in PBMCs in patients with active KS compared with those with KS in remission,[79] and KSHV levels in buffy coat cells were higher in those patients with higher rates of eruptions of KS lesions.[80] A study comparing plasma and PBMC KSHV load in patients with KS found that there was generally a linear relationship between the two,[78] although there was significant variation in the correlation for many individuals. Despite the detection of KSHV in the blood of KS patients and apparent correlation with KSHV load and disease activity, the clinical use of viral loads for monitoring KS activity or as a guide for therapy is limited by the relatively low levels of KSHV viremia.[79] In contrast, the levels of KSHV viremia are significantly higher in multicentric Castleman disease.

## Genetic Predisposition to KS

Rare genetic disorders can predispose to KS. Wiskott-Aldrich syndrome, caused by a mutation of the *WAS* gene, is X-linked recessive, results in susceptibility to many infections, and can lead to aggressive KS. Interferon-γ receptor 1 deficiency, caused by mutation in the *IFNGR1* gene, is autosomal recessive and also results in a generalized immunodeficiency that can lead to KS.[6] Mutation of *STIM1,* which encodes the stromal interaction molecule 1, results in an autosomal-recessive disorder, and had led to fatal KS in a 2-year old child.[81] STIM1 is an endoplasmic reticulum membrane protein, which regulates calcium stores in the cell, and defects in this gene also result in susceptibility to other infections.[82] Mutation of the *TNFRSF4* gene, encoding OX40, leads to an autosomal-recessive disorder that was described in a 14-year-old patient who developed aggressive KS.[7] This patient did not have a history of other infections. A rare heterozygous amino acid substitution in the *STAT4* gene was present in five individuals with KS in one family; these family members did not otherwise have a history of immunodeficiency.[8] The unifying theme for these genetic disorders is a defect in T-cell immunity, leading to loss of control of KSHV infection. Case-control studies have also identified genetic variants of *FCGR3A, CXCR2,* and *IL13* that may be linked with classic KS.[2]

## Treatment

ART is generally recommended for HIV-infected patients with KS and often leads to regression of KS lesions as the immune system reconstitutes.[65,83] In iatrogenic KS, boosting immunity through reduction of immune suppression can lead to KS remission, again highlighting the critical role of the immune response to KSHV. KS regressed in renal transplantation patients with KS who were switched from cyclosporine to rapamycin (sirolimus) immunosuppression. Therefore immunosuppression with rapamycin (or one of its analogues) should be considered in transplant recipients with KS.[84,85]

Despite these approaches, targeted therapy of KS is often necessary. Such strategies are palliative and not curative.[65,69,75,83] Depending on the severity of disease, treatment options may include observation, topical therapy, or systemic therapy. Local therapy may include chemotherapeutic agents, laser treatment, cryotherapy, and irradiation. Systemic therapy is reserved for more severe disease and includes liposomal anthracyclines, paclitaxel, and vinorelbine. Importantly, corticosteroids have been associated with the appearance or worsening of KS lesions. Withdrawal or reduction of steroids can lead to regression of KS lesions.[9]

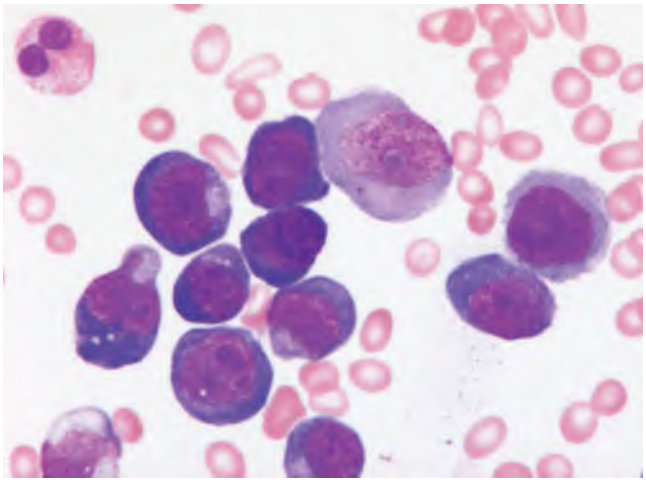

**FIG. 140.3 Kaposi sarcoma–associated herpesvirus–infected primary effusion lymphoma cells from this pleural effusion have plasmacytoid features and deeply basophilic cytoplasm.** Red blood cells are interspersed among the lymphoma cells. (Wright-Giemsa stain, ×1000.) *(Courtesy Dan Jones, MD, PhD.)*

## KS-Associated IRIS After Institution of Antiretroviral Therapy

With the increased utilization of combined ART, the phenomenon of IRIS has also been recognized in the setting of KS. Manifestations have included worsening of KS that is already clinically present or "unmasking" of KS that was not apparent until then.[86–88] Therapy with ART should generally be continued in cases of KS-associated IRIS, and cytotoxic chemotherapy may also be necessary to control disease.[89–91]

## Primary Effusion Lymphoma

PEL was first described in 1989 in HIV-infected patients.[92] It occurs in the potential body spaces of the pleural, pericardial, and peritoneal cavities.[9,93] Lymphoma cells (Fig. 140.3) grow in suspension with little or no contiguous solid mass component. Cells contain clonal immunoglobulin gene arrangements, indicating a B-cell origin, despite lacking most typical B-cell antigens. The malignant cells are infected with KSHV, and Epstein-Barr virus often coinfects the cells. PEL is rare, accounting for approximately 3% of AIDS-related lymphomas and only an estimated 0.4% of non–AIDS-associated large cell non-Hodgkin lymphomas.[94] The prognosis is poor, with death often occurring within months of diagnosis. Patients with PEL tend to have higher levels of KSHV viremia than those with KS but lower levels than patients with multicentric Castleman disease.[79,95] In HIV-associated PEL, ART appears to be beneficial and is typically administered with cytotoxic chemotherapy. Radiation can sometimes be used when chemotherapy is not possible or has failed.[10] A rare solid tumor variant of PEL that does not occur in potential body cavities has also been described in HIV-infected patients.[96]

## Multicentric Castleman Disease

Castleman disease is a rare lymphoproliferative disorder, first described in 1956,[97] that occurs in two forms. Localized Castleman disease (hyaline vascular variant) is not associated with KSHV and has an indolent clinical course. Multicentric Castleman disease (plasma cell variant), first described in 1978,[98] is associated with KSHV and has a much more aggressive clinical course, frequently resulting in death. Multicentric Castleman disease is often associated with fever, hepatosplenomegaly, and generalized lymphadenopathy. Complications include infection (often a cause of death) and the development of either a plasmablastic lymphoma or KS.[99,100] KSHV is almost always linked to multicentric Castleman disease in HIV-infected individuals, and KSHV infection is linked to approximately 50% of cases in individuals without HIV infection.[101,102] IL-6, which induces B-cell differentiation, is expressed at high levels in the germinal centers of affected lymph nodes and may be responsible for the high numbers of plasma cells

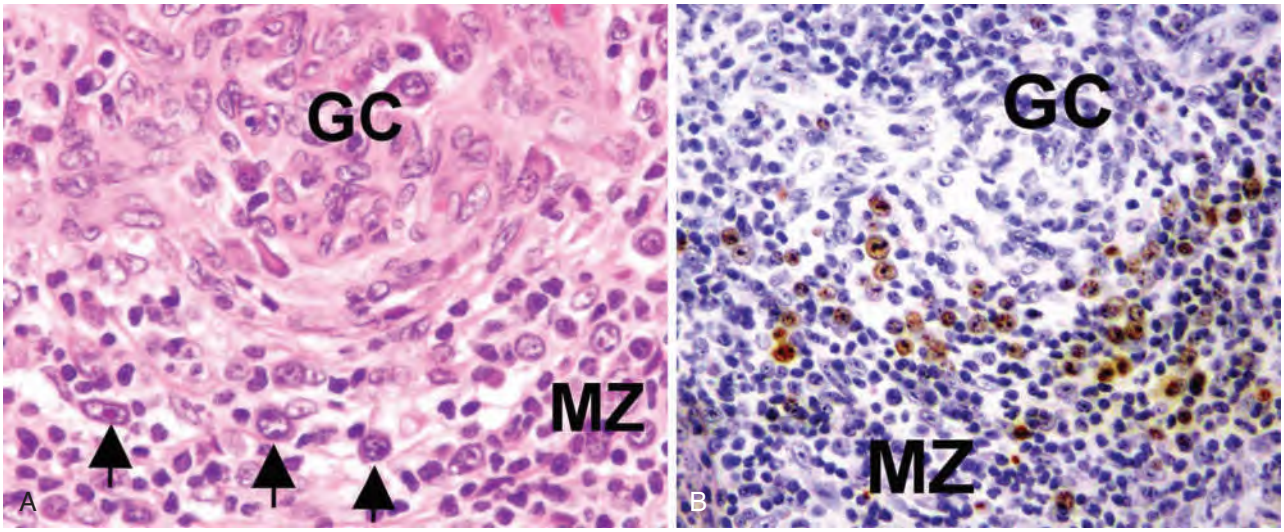


**FIG. 140.4 Multicentric castleman disease in a lymph node of a human immunodeficiency virus–negative patient.** (A) Regressed germinal center *(GC)* has atypical plasmablasts *(arrows)* concentrated in the follicle mantle zone *(MZ)*. (Hematoxylin and eosin stain, ×600.) (B) Immunohistochemical detection of Kaposi sarcoma–associated herpesvirus (KSHV) latency-associated nuclear antigen *(brown)* in the nuclei of plasmablasts indicates KSHV-infected cells (×400). *(Courtesy Dan Jones, MD, PhD.)*

present (Fig. 140.4A). Of interest, KSHV encodes a homologue of IL-6 that is expressed in latent and lytic infection and may have a role in disease.[103,104] KSHV-infected plasmablasts are typically seen in the mantle zone of affected lymph nodes (Fig. 140.4B).[105] Although the optimal therapy for multicentric Castleman disease is not clearly defined, treatment modalities include the anti-CD20 monoclonal antibody rituximab, a monoclonal antibody directed against the IL-6 receptor (tocilizumab) or against IL-6 (siltuximab), ganciclovir, and cytotoxic chemotherapy.[100,106–108]

Of interest, KSHV viral loads in peripheral blood are relatively high in multicentric Castleman disease (ranging up to 4 or 5 logarithms). High KSHV levels can be found in both PBMCs and plasma.[96] Furthermore, the presence of symptoms or active disease has been associated with higher levels of KSHV in PBMCs compared with the absence of symptoms or when disease is in remission.[79,109] For this reason, KSHV viral loads may be useful for monitoring activity of disease during treatment of patients with multicentric Castleman disease.[79] It is unclear why there are higher levels of KSHV DNA in multicentric Castleman disease compared with KS. It is possible that there may be increased levels of lytic replication occurring in patients with multicentric Castleman disease,[79,110] but this question remains open.

### KSHV Inflammatory Cytokine Syndrome

Recently, a manifestation of KSHV has been described in HIV-infected individuals that appears to be distinct from multicentric Castleman disease.[111] This disease, designated KSHV inflammatory cytokine syndrome (KICS), clinically resembles multicentric Castleman disease in a number of respects, including the presence of systemic inflammation, but the pathologic lymph node findings are absent. KICS patients typically have KS and may also have PEL. In a prospective study, patients diagnosed with KICS had more severe symptoms, higher KSHV viral loads, and an increased risk of death compared with HIV-infected and HIV/KSHV-coinfected non-KICS control subjects.[112,113] Severe CD4 lymphocytopenia appears to be more common in KICS patients, and it is possible that the pathologic characteristics of multicentric Castleman disease are less likely to develop in the setting of very low CD4 T-cell counts.

### Other Syndromes

A number of syndromes have been linked to KSHV infection but either are disputed in the literature or have not been confirmed. These include the skin diseases pemphigus and bullous pemphigoid, sarcoid, Kikuchi disease, multiple myeloma, hemophagocytic syndrome, and primary pulmonary hypertension.[9,114] An intriguing link between KSHV and ketosis-prone diabetes has been observed.[115]

## THERAPY AND PREVENTION

Several agents have activity against KSHV lytic replication, but none has an established role in KSHV-associated diseases. Ganciclovir, foscarnet, cidofovir, and adefovir, but not acyclovir, inhibit KSHV lytic replication.[116–119] A likely reason for a lack of efficacy of these agents in KSHV-associated diseases is that they target lytic, rather than latent, replication of KSHV. The vast majority of KSHV-infected cells in KS, PEL, and multicentric Castleman disease are latently, not lytically, infected. Development of agents that target latent infection would therefore likely result in a major advance in treatment of KSHV-associated diseases.

Lytic KSHV infection has a role in the biology and transmission of KSHV. Of note, a study investigating cytomegalovirus retinitis in AIDS showed that ganciclovir reduced the incidence of KS.[120] Also, a randomized study showed that oral valganciclovir reduced oropharyngeal KSHV shedding,[121] indicating that interference with lytic infection might reduce rates of KSHV transmission. However, although valganciclovir was well tolerated in this study, the adverse effects of ganciclovir or valganciclovir would mitigate against either being used widely for the prevention of KSHV transmission or disease. The best prevention of KSHV-associated disease would be a vaccine to prevent infection or the development of malignancy, but to date no vaccine has been developed.

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*The complete reference list is available online at Expert Consult.*

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# 141 Herpes B Virus

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## SHORT VIEW SUMMARY

**Definition**
- Herpes B virus is a macaque virus that can cause fatal encephalitis in humans.

**Epidemiology**
- Herpes B virus naturally infects Old World macaques, including rhesus and pig-tailed macaques and cynomolgus monkeys.
- Humans are infected with herpes B virus after bites, scratches, needlesticks, or mucosal splashes with fluids from Old World macaques.

**Microbiology**
- Herpes B virus is an alphaherpesvirus and is the homolog of herpes simplex virus in macaques.

**Diagnosis**
- A positive polymerase chain reaction (PCR) or culture for herpes B virus in skin lesions, conjunctival swabs, or cerebrospinal fluid in the presence of symptoms is diagnostic for herpes B virus infection.
- A positive PCR or culture for herpes B virus of wounds or mucosa shortly after injury indicates exposure to the virus but not necessarily infection.
- Human specimens for PCR, culture, or antibody testing should be sent to the National B Virus Resource Center in Atlanta, Georgia (www2.gsu.edu/~wwwvir/index.html).

**Therapy**
- Individuals with signs or symptoms of B virus or positive cultures or PCR (other than wound or postcleansing PCR or culture) and exposure to Old World macaques should be treated.
- Intravenous acyclovir (12.5–15 mg/kg q8h) or ganciclovir (5 mg/kg every 12 hours) is recommended for patients without central nervous system diseases (CNS) disease.
- Intravenous ganciclovir (5 mg/kg every 12 hours) is recommended for patients with CNS disease.
- Treatment is continued until symptoms resolve and two cultures over a 2-week period are negative; oral valacyclovir or acyclovir is often given after intravenous therapy is discontinued to prevent reactivation of latent virus.

**Prevention**
- First aid, with thorough cleansing of wounds or exposed mucosa, is important after injuries or mucosal splashes with macaque fluids.
- Individuals who have high risk of exposure to herpes B virus (see Table 141.1) should receive postexposure prophylaxis within 5 days of exposure with valacyclovir, 1 g three times a day, or acyclovir, 800 mg five times daily, for 14 days.

Herpes B virus, whose taxonomic species name was formerly Cercopithecine herpesvirus 1 and is now Macacine herpesvirus 1,[1] causes a disease in macaque monkeys that is similar to that seen with herpes simplex virus (HSV) type 1 in humans; however, infection of immunocompetent humans with herpes B virus can result in fatal encephalitis. Individuals who are scratched or bitten or have splashes to mucosal surfaces with material from macaque monkeys should be evaluated for possible herpes B virus infection and, when appropriate, should receive postexposure prophylaxis or treatment.

## HISTORY

Herpes B virus was first described in 1933[2] in a researcher who died after being bitten by a macaque. Sabin and Wright[3] isolated the virus and named it B virus after the patient's last name. About 50 cases of herpes B virus in humans have been reported in the literature, with 26 well-documented cases.[4]

## DESCRIPTION OF THE VIRUS

Herpes B virus is an alphaherpesvirus in the same subfamily as HSV. The complete sequence of herpes B virus[5] shows that it is closely related to HSV with a conserved genomic structure, and the viral glycoproteins show about 50% amino acid identity between the two viruses.

## EPIDEMIOLOGY

Herpes B virus is endemic in Old World macaques, and most macaques in captivity (unless separated from their parents at birth and reared apart from other animals) should be considered as possibly infected. Most macaques are infected during adolescence, and nearly 100% of adult (≥2.5 years old) macaques bred in captivity or in the wild are infected.[6] The virus naturally infects all types of Old World macaques including rhesus macaques *(Macaca mulatta),* cynomolgus monkeys *(Macaca fascicularis),* and pigtailed macaques *(Macaca nemestrina).* The virus has also been isolated from bonnet, Japanese, stumptail, and other macaques, but no other Old World or New World monkeys are naturally infected.[7] Herpes B virus has also been detected in free-ranging monkeys in Bali and other sites in Southeast Asia.[8]

Humans are inadvertent hosts. Humans have been infected by bites and scratches from macaques. Other exposures that have transmitted the virus are needlestick injury from a needle that was exposed to tissue around the eye of a macaque or a needle that was thought to be used to inject monkeys, contamination of wounds with macaque saliva, lacerations from bottles containing macaque cell cultures, scratches from cages, exposure to monkey nervous tissue at autopsy, and possible aerosol exposures.[4] One reported case was caused by a splash to the eye from material from a caged macaque.[9] A single case of human-to-human transmission of herpes B virus was reported in a woman who became infected after applying hydrocortisone cream to her contact dermatitis lesions and her husband's herpes B virus skin lesions.[10] Herpes B virus was reported in a primate worker who had not cared for primates for more than 10 years. The disease was presumed to be due to reactivation of the virus from latency in the worker[11]; however, this case is considered controversial, and the patient may have had an unrecognized exposure to herpes B virus more recently. All cases of herpes B virus except for the patient with mucosal splash have been due to percutaneous exposure. Although a large number of animal bites and scratches occur each year, cases of herpes B virus are rare; nonetheless, the potential for fatalities requires that each of these exposures be evaluated.

**FIG. 141.1 Macaque with a lesion on the upper lip due to herpes B virus infection.** *(Courtesy J. Hilliard, Georgia State University, Atlanta, GA.)*

## PATHOGENESIS

Animals are infected through the mucosa or skin from oral or genital secretions of other animals. Herpes B virus rarely causes disease in macaques,[12] although oral lesions can occur (Fig. 141.1). The virus is latent in the sensory ganglia of the animals and can reactivate with shedding. Sites of shedding include the genital tract and oral and conjunctival mucosa. One study reported oropharyngeal or urogenital shedding rates of 39% in macaques shortly after capture and shipping.[13] On a given day, about 2% of herpes B virus–seropositive healthy adult monkeys shed virus.[14] Shedding is more common in animals that are ill, immunocompromised, stressed, or breeding. Similar to HSV in humans, latently infected macaques shed virus intermittently and often in the absence of lesions. Peripheral blood of macaques has been reported to contain herpes B virus in animals that are ill[15]; viremia rarely, if ever, occurs in healthy macaques.[16]

Humans are infected from monkey oral, genital, or ocular secretions or monkey nervous system tissues, with a usual incubation period of 5 days to 3 weeks (range, 2 days to 5 weeks). The virus replicates at the site of infection and then ascends the peripheral nervous system in retrograde fashion before advancing to the central nervous system (CNS). Antibody to HSV does not protect humans from herpes B virus infection.

## CLINICAL MANIFESTATIONS

Asymptomatic infection (i.e., seropositivity without disease) of human primate workers with herpes B virus, including most of those who had histories of bites and scratches, has not been detected.[17,18] Most human infections have been reported from animals without any symptoms. Infection of humans with herpes B virus can initially manifest in three different forms. First, patients may present with nonspecific flulike symptoms including fever, chills, myalgias, and malaise before presenting with CNS symptoms. Second, patients may present with symptoms including itching, tingling, numbness, and pain at the site of herpes B virus inoculation. Some patients have a vesicular rash at the inoculation site and may have lymphadenopathy in the draining lymph nodes. Third, patients may present directly with peripheral nervous system or CNS symptoms. Patients with the first two presentations may develop weakness or paresthesias involving the nerve at the site of infection before developing CNS symptoms. These symptoms include headache, nuchal rigidity, nausea, vomiting, confusion, dysphagia, dysarthria, ataxia, urinary retention, and cranial nerve palsies. The disease progresses from the upper spinal cord to the brainstem and then results in a global encephalitis manifested by seizures, ascending paralysis, hemiplegia, coma, and respiratory failure. Additional symptoms include sinusitis, conjunctivitis, hiccups, and abdominal pain. The mortality rate in untreated humans is estimated at 70%[7] and is considerably lower in patients treated at an early stage of the disease.

## LABORATORY DIAGNOSIS AFTER EXPOSURE

Some authorities recommend obtaining baseline serum at the time of exposure to simultaneously test it with serum obtained about 3 to 6 weeks later to document seroconversion or a fourfold rise in titer. Patients receiving acyclovir may have delayed seroconversion; serum can be obtained from patients receiving postexposure prophylaxis 3 to 6 weeks after the exposure and at 12 weeks. Because asymptomatic infection has never been reported, other authorities do not recommend testing serum except to confirm a diagnosis in individuals with symptoms compatible with herpes B virus disease. Positive serologies are confirmed using competition enzyme-linked immunosorbent assay or Western blotting.[17]

Cultures of the wound or exposed mucosa should be obtained only after cleansing is performed so as not to delay first aid or removal of virus from the site. Some authorities believe that cultures are not especially helpful because decisions must be made regarding postexposure prophylaxis before the results are available. Any patient who has a positive culture for herpes B virus needs subsequent follow-up cultures to ensure that they are not shedding virus.

A polymerase chain reaction (PCR) assay for herpes B virus DNA can be performed on lesion swabs,[19] spinal fluid,[20] and other sites. A positive PCR in the setting of symptoms consistent with herpes B virus is considered diagnostic of infection.

Some authorities recommend testing the primate with which the patient was in contact for herpes B virus by culture or serologic test. However, the monkeys can be in the process of seroconverting at the time of the exposure, and a positive serologic test in a monkey does not indicate that it is actively shedding virus. Culture, serology, and PCR testing of humans and primates in the United States is performed by the National B Virus Resource Center in Atlanta, Georgia; the virus should be isolated only in a BSL-3 laboratory. Their website (www2.gsu.edu/~wwwvir/index.html) offers useful information on collecting and shipping specimens.

## POSTEXPOSURE EVALUATION AND PROPHYLAXIS

First aid, with prompt, thorough irrigation of wounds and exposed mucosal tissues, is essential to reduce the likelihood of infection. Mucous membranes should be flushed with saline, and wounds should be irrigated with detergent (e.g., chlorhexidine or povidone-iodine) for 15 minutes.[4,21] A health care professional should evaluate the inoculation site and thoroughness of cleansing, document the type of exposure (including whether it involved a macaque), consider obtaining baseline serum samples, consider culturing the wound, educate the patient regarding signs and symptoms of herpes B virus, identify a local medical consultant if the need arises, and consider postexposure prophylaxis. The medical history of the monkey should be evaluated, including whether it is ill or immunocompromised or has lesions compatible with herpes B virus infection. All these factors increase the risk that the animal is actively shedding herpes B virus.

Postexposure prophylaxis with oral acyclovir or ganciclovir was shown to be effective in a rabbit model of herpes B virus infection[22,23] but has not formally been shown to be effective in humans. Nonetheless, although postexposure prophylaxis with antiviral therapy has been recommended only since 1995,[7] no cases of herpes B virus have been reported to date in people receiving postexposure prophylaxis within 3 days of exposure.[4]

A working group convened in 2002 by the US Centers for Disease Control and Prevention prepared a series of recommendations for postexposure prophylaxis of herpes B virus.[4] Certain types of exposure to macaques were considered to impart a much higher risk of herpes B virus infection in humans. These include inadequately cleansed wounds, deep puncture wounds (which are difficult to clean), bites to the head and face (in which virus can quickly travel to the CNS), exposures involving materials known or highly likely to be infected with herpes

**TABLE 141.1 Recommendations for Postexposure Prophylaxis for Persons Exposed to Herpes B Virus**

| |
|---|
| **Prophylaxis Recommended** |
| Skin exposure[a] (with loss of skin integrity) or mucosal exposure (with or without injury) to a high-risk source (e.g., a macaque that is ill, immunocompromised, or known to be shedding virus or that has lesions compatible with herpes B virus disease) |
| Inadequately cleaned skin exposure (with loss of skin integrity) or mucosal exposure (with or without injury) |
| Laceration of the head, neck, or torso |
| Deep puncture bite |
| Needlestick associated with tissue or fluid from the nervous system; lesions suspicious for herpes B virus; eyelids or mucosa exposure |
| Puncture or laceration after exposure to objects (a) contaminated either with fluid from monkey oral or genital lesions or with nervous system tissues or (b) known to contain herpes B virus |
| A postcleansing culture is positive for herpes B virus |
| **Prophylaxis Considered** |
| Mucosal splash that has been adequately cleaned |
| Laceration (with loss of skin integrity) that has been adequately cleaned |
| Needlestick involving blood from an ill or immunocompromised macaque |
| Puncture or laceration occurring after exposure to (a) objects contaminated with body fluid (other than that from a lesion) or (b) potentially infected cell culture |
| **Regimen for Prophylaxis** |
| Valacyclovir, 1 g PO tid, or acyclovir, 800 mg PO 5 times daily × 14 days |
| **Prophylaxis Not Recommended** |
| Skin exposure in which the skin remains intact |
| Exposure associated with nonmacaque species of nonhuman primates |

[a]Exposures include macaque bites or scratches or contact with ocular, oral, or genital secretions; nervous system tissues; or materials contaminated by macaques (e.g., cages or equipment).
*PO,* Per os (orally).
*From Cohen JI, Davenport DS, Stewart JA, et al. Recommendations for prevention and therapy of persons exposed to B virus (Cercopithecine herpesvirus 1).* Clin Infect Dis. *2002;35:1191–1203.*

B virus, and exposures involving ill or immunocompromised macaques or those with lesions consistent with herpes B virus disease. Recommendations concerning which patients should receive postexposure prophylaxis are presented in Table 141.1. Postexposure prophylaxis is given as early as possible and within 5 days of the exposure because animals given antiviral medication have benefited as late as 5 days after inoculation.[22,23] Postexposure prophylaxis is not a substitute for prompt and thorough cleansing of the infected site. Most authorities recommend either valacyclovir, 1 g three times daily, or acyclovir, 800 mg five times daily for 14 days, although these medications are not approved for this use by the US Food and Drug Administration. High doses of the oral drugs are used because the dose needed to inhibit virus replication by 50% for herpes B virus is 18 μg/mL,[23] which is about 10 times higher than that for HSV. Valacyclovir is the drug of choice because of the higher serum levels of acyclovir achieved with valacyclovir than with oral acyclovir. If symptoms compatible with herpes B virus disease occur while patients are receiving postexposure prophylaxis, treatment for herpes B virus should be started; thus it is important to follow up patients with a potential herpes B virus exposure whether or not they receive postexposure prophylaxis.

## DIAGNOSIS OF HERPES B VIRUS DISEASE

A physical examination of the lesion site (looking for vesicles) and a complete neurologic examination should be performed in patients with herpes B virus disease. Cultures of conjunctiva, oropharynx, and the exposure site are recommended, along with obtaining serum for herpes B virus serologic testing. Magnetic resonance imaging of the brain should be performed, and cerebrospinal fluid should be sent for a PCR assay.[7,20,24,25] Electroencephalography may help differentiate herpes B virus, which initiates with upper spinal cord and brainstem involvement and results in a diffuse encephalitis, from HSV encephalitis, which usually involves one of the temporal lobes. Somatosensory evoked potentials can help identify early lesions in the brain or spinal cord.

PCR assay for herpes B virus has been reported using primers for glycoprotein G, which differs from glycoprotein G in HSV-1 and HSV-2.[25] A real-time PCR assay was as specific, but twice as sensitive, as culture to detect herpes B virus in human and monkey specimens. Regions on herpes B virus glycoproteins B and D have been identified that are recognized by antibodies in sera from infected macaques, and these are being studied for future peptide-based serologic assays to detect herpes B virus infection.[26]

## THERAPY

Intravenous treatment rather than oral prophylaxis should be initiated in any patient with signs or symptoms of herpes B virus or a positive culture or PCR (not including a postcleansing culture or PCR from the wound) if the patient has had a documented exposure to a macaque. In the absence of CNS symptoms, either acyclovir, 12.5 to 15 mg/kg intravenously every 8 hours, or ganciclovir, 5 mg/kg intravenously every 12 hours, is recommended until symptoms resolve and two cultures over a 2-week period are negative for herpes B virus.[4] Because in vitro studies and animal models show that herpes B virus is more sensitive to ganciclovir than acyclovir,[23,27] most experts recommend ganciclovir for patients with CNS symptoms. If herpes B virus can establish latency and reactivate in humans, discontinuation of antiviral therapy could allow reactivation to occur. Therefore many authorities recommend that patients who survive herpes B virus infection be maintained on oral acyclovir or valacyclovir, initially at doses used for postexposure prophylaxis and later at suppressive doses, for a prolonged time after intravenous therapy is stopped.[4,7] Repeated cultures for herpes B virus are often recommended after intravenous therapy has been changed to oral therapy to confirm that herpes B virus shedding is not occurring or when antiviral therapy is discontinued.

Before antiviral therapy was available, about 80% of patients with herpes B virus infection died; with antiviral therapy, it is estimated that 80% of patients survive.[28] Although there have been relatively few cases of documented herpes B virus infection treated in the era of antiviral therapy, five patients with laboratory-confirmed infection with herpes B virus (some of whom had CNS symptoms) who were treated with intravenous acyclovir or ganciclovir had resolution of symptoms within 2 to 3 weeks of therapy.[10,18,29,30] As with HSV encephalitis, therapy for herpes B virus encephalitis is likely to be more effective when given earlier.

## PREVENTION

Prevention of herpes B virus requires strict precautions when working with nonhuman primates. In view of a fatal case of herpes B virus occurring in a woman who received a splash to her eyes, primate workers exposed to macaques should wear goggles or glasses with side shields and a mask or a chin-length face shield and a mask to prevent infection of the eyes and oral mucosa.[4] Although only a single case of person-to-person transmission of herpes B virus has been reported,[10] individuals infected with the virus can shed infectious virus for more than 1 week even while receiving intravenous acyclovir[4]; therefore body fluids should be considered potentially infectious. Oral and genital secretions from individuals who have been exposed to herpes B virus, when it is not yet known whether they are infected, should be considered potentially infectious to others. If the incubation period for herpes B virus (generally 5 weeks in untreated individuals) has passed and the person is asymptomatic or serologies are persistently negative (at least 12 weeks after exposure in patients given antiviral prophylaxis), the likelihood of infection and virus transmission is exceedingly low.

It is essential that individuals exposed to macaques be educated regarding the importance of first aid and the need for rapid cleansing of wounds or mucosal exposures, the need to see health care personnel regarding evaluation for postexposure prophylaxis, and the signs and symptoms of herpes B virus disease so that early therapy can be initiated. Specific pathogen-free colonies of macaques have reduced the risk of herpesvirus B virus exposure almost 20-fold.[31]

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*The complete reference list is available online at Expert Consult.*


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# 142 Adenoviruses

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## SHORT VIEW SUMMARY

### Definition
- Human adenoviruses (HAdVs) are DNA viruses that can cause a broad range of clinical syndromes, including respiratory tract infections, ocular disease, gastroenteritis, diarrhea, and cystitis.

### Epidemiology
- HAdVs are ubiquitous; most humans have serologic evidence of prior infection by age 10 years.
- Transmission is via respiratory droplets or fecal-oral transmission. Virus secretion may persist for prolonged periods after acute infection resolves.
- Typically, the disease is subclinical or mildly symptomatic and self-limited.
- HAdVs are a common cause of febrile illness, respiratory tract infections (types 1–7), and gastroenteritis (types 2–5) in young children; sporadic pediatric outbreaks are associated with daycare centers and summer camps.
- Acute respiratory disease, including pneumonia, is uncommon; sporadic outbreaks are associated with military recruits (types 4 and 7); a recent outbreak occurred in healthy adults (type 14).
- Epidemic keratoconjunctivitis (types 8, 19, and 37) has been linked to nosocomial transmission by infected instruments.
- HAdV is an emerging opportunistic pathogen in immunocompromised hosts, primarily hematopoietic stem cell transplants (HSCT) and solid-organ transplant (SOT) recipients; it can result in disseminated disease or target grafted organ.
- There is interest in using HAdVs as vectors for gene therapy, in vaccines being developed for infectious diseases (human immunodeficiency virus, Ebola, Zika); and as immunomodulatory treatments for solid tumors.

### Microbiology
- HAdVs are nonenveloped, lytic DNA viruses, characterized by serologic responses to major capsid proteins and whole-genome analysis.
- HAdVs were originally isolated from adenoid tissues, leading to the virus name.
- HAdVs are classified into 7 species (A–G); 85 types have been detected in clinical specimens so far.

### Diagnosis
- Diagnosis is not routinely pursued because most infections are mild and self-limited.
- HAdVs can be isolated by routine viral tissue culture (except types 40 and 41) and can be recovered from swabs, samples, and tissues; immunofluorescence assay, enzyme-linked immunosorbent assay, acute/convalescent serum titers can establish diagnosis.
- Polymerase chain reaction assays for HAdVs are highly sensitive and specific (96%–100%) and are now widely used.

### Therapy
- There are no approved therapies available.
- Case series report partial clinical response but substantial toxicities to cidofovir in HSCT/SOT. Clinical trials of brincidofovir in transplant patients are underway.

### Prevention
- Live oral vaccines (type 4 and 7) are administered to military personnel and are highly effective in preventing adenovirus-associated febrile respiratory diseases.

In 1953 Rowe and coworkers[1] isolated a novel cytopathic agent from surgical human adenoid samples undergoing spontaneous degeneration in tissue culture. Soon after, Hilleman and Werner[2] recovered similar viral agents from cases of acute respiratory disease (ARD) in military personnel. To denote their origin, these agents were designated adenoviruses. Subsequently, links to clinical disease were established by studies in which rising antiadenovirus antibody titers were detected in historic serum samples from World War II military personnel and from patients with ARD, exudative tonsillitis, and atypical pneumonia.[3] In 1955 adenovirus type 8 was identified as a cause of epidemic keratoconjunctivitis.[4]

In the 20 years after the discovery of adenoviruses by Rowe and coworkers, more than 30 different adenovirus types were identified and were shown to cause several clinical syndromes, including upper and lower respiratory tract infections, keratoconjunctivitis, and infantile gastroenteritis.[5] Epidemiologic studies in the 1960s and 1970s established that adenovirus infections are very common, causing 5% to 10% of all febrile illnesses in infants and young children.[6] Although clinically evident adenovirus infections are typically mild and self-limited in immunocompetent patients, outbreaks of severe respiratory disease associated with significant morbidity and occasional deaths have been observed in neonates and military recruits[7,8,9] and, more recently, in civilian populations.[7,10] Adenoviruses have also emerged as serious opportunistic pathogens in immunocompromised patients who have undergone hematopoietic stem cell or solid-organ transplantation (SOT).[11,12] With the advent of molecular diagnostics and application of whole-genome sequence analysis, at least 85 human adenovirus types have been identified.[13–15] The original 51 HAdV types were determined by serology, and types 52 to 85 were determined by whole-genome sequencing and phylogenomics, with serology as an adjuvant for some types.[16] The original 51 serotypes are also recognized as individual genotypes by whole-genome sequencing. Although several adenovirus types have not been linked to clinical disease, many have been shown to cause a broad range of clinical syndromes, including hepatitis, hemorrhagic cystitis, nephritis, myocarditis, and meningoencephalitis.

In 1962 adenovirus type 12 was shown to cause tumors in rodent cells.[17] This was the first description of a human virus that could induce malignant tumors in animals, and certain adenoviruses became model systems for studying oncogenesis. However, the oncogenic potential of adenoviruses has not been associated with any malignancies in humans. Adenoviruses also provided an important model system for studying viral and cellular gene expression and regulation, cell-cycle control, and DNA replication.[18] Recently, intense interest has focused on using

modified adenoviruses as vectors for gene therapy and vaccines for infectious diseases and cancers.

## DESCRIPTION OF THE PATHOGEN

Adenoviruses are nonenveloped, lytic DNA viruses. Mature virions are 70 to 90 nm in diameter and contain a linear 36-kilobase double-stranded DNA core complex encased in an icosahedral capsid (Fig. 142.1). The adenovirus capsid is composed primarily of three major capsid proteins called *hexon, penton*, and *fiber* (Fig. 142.2). There are 252 subunits called *capsomeres*, including 240 hexon proteins and 12 penton proteins that form the 20 surfaces and 12 vertices of the capsid. At each vertex a penton protein is located at the base from which a fiber protein protrudes. The fiber protein interacts with primary cellular receptors and consists of a distal globular knob, a central shaft, and a tail that anchors the wandlike fiber to its penton base.[18–20] Although the overall structure of adenoviruses appears to be conserved across serotypes, the length of the fibers can vary and is specific for a given type.[21] Less well characterized are several minor proteins, including IIIa, VI, VIII, and IX, that contribute to stabilization of the capsid structure. Most of the epitopes recognized by group- and type-specific antibodies are present on the hexon and fiber proteins. Hexon proteins contain seven short hypervariable regions that are located on the solvent-exposed surface and that represent type-specific targets of dominant neutralizing antibodies. Fiber proteins also contain certain type-specific antigenic determinants that are responsible for in vitro hemagglutination characteristics.[22]

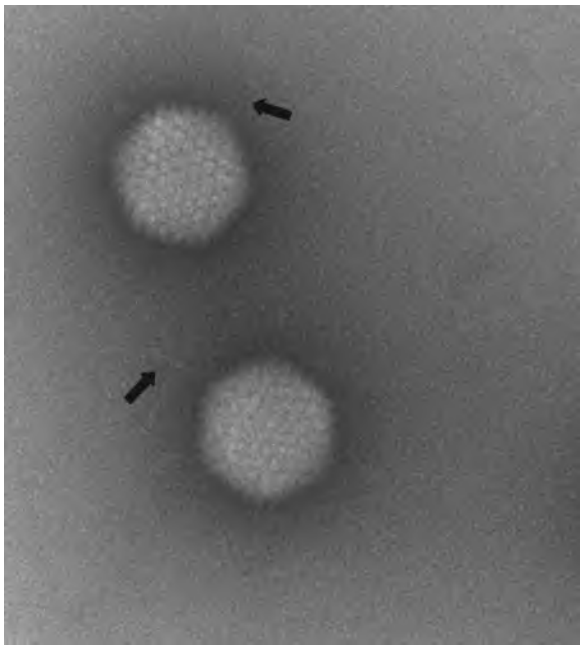

**FIG. 142.1 Electron micrograph of adenovirus particles.** Arrows indicate fibers.

More than 100 different adenoviruses have been isolated from vertebrates, ranging from reptiles to humans. Nonhuman adenoviruses have not been demonstrated to cause clinical disease in humans. Human adenoviruses belong to the genus *Mastadenovirus* (encompassing all mammalian adenoviruses) and are further divided into seven species (A–G) based on their hemagglutination characteristics (Table 142.1). Further characterization into types has been determined in part by their resistance to neutralization by antibodies to other known adenoviruses.[23] Revised criteria for typing human adenoviruses now include genome sequence data and computational analysis in addition to traditional serologic criteria, reflecting recent approaches to characterizing novel adenoviruses.[24,25] Adenoviruses have also been classified by their oncogenic properties, including their ability to transform cells in cultures and cause tumors in animals, and by the percentage of guanine and cytosine in adenovirus DNA.[18]

## INTERACTIONS WITH THE HOST

Adenoviruses can cause a broad range of clinical syndromes, but it is not well understood why specific adenovirus types are often associated with particular syndromes. The portal of viral entry often appears to determine the primary site of disease, as seen in the spread of ARD by respiratory droplets or of infantile diarrhea by fecal-oral transmission, whereas other organ-limited diseases, such as hemorrhagic cystitis, likely result from a viremic phase of infection. Tissue tropism varies between different adenovirus species; species C, E, and some B viruses typically infect the respiratory tract; species D viruses can cause ocular and gastrointestinal (GI) infections; and species A, F, and G viruses target the GI tract. Viral tropism may be partially determined by differences in virus binding and host cell entry, which is typically initiated by binding of the fiber knob to a high-affinity receptor on the cell surface. Internalization of the virus particle is then mediated by association of the penton base with cell surface integrins.[26]

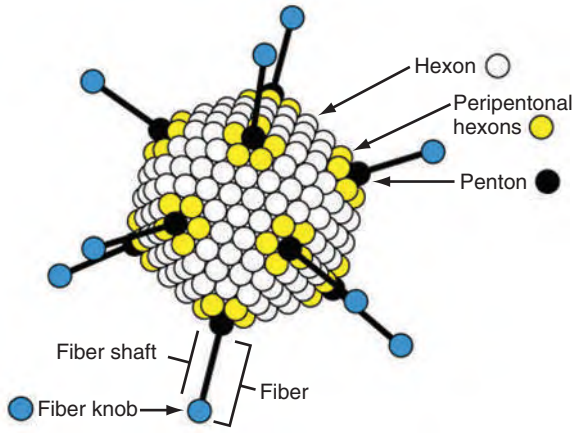


**FIG. 142.2 Schematic of an adenovirus capsid.**

**TABLE 142.1 Classification of Adenoviruses**

| GROUP | HEMAGGLUTINATION GROUPS | TYPES | COMMON SITES OF INFECTION |
|---|---|---|---|
| A | IV (little or no agglutination) | 12, 18, 31 | GI tract, respiratory tract |
| B | I (complete agglutination of monkey erythrocytes) | 3, 7, 11, 14, 16, 21, 34, 35, 50, 55 | Respiratory tract, genitourinary tract |
| C | III (partial agglutination of rat erythrocytes) | 1, 2, 5, 6, 57 | Respiratory tract, liver |
| D | II (complete agglutination of rat erythrocytes) | 8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51, 53, 54, 56, 58–60 | Eye, GI tract |
| E | III | 4 | Respiratory tract |
| F | III | 40, 41 | GI tract |
| G | III | 52 | GI tract |

*GI,* Gastrointestinal.

The primary cellular receptor for the majority of adenoviruses, including species A, C, E, and F adenoviruses, is the coxsackie B virus–adenovirus receptor (CAR), a transmembrane protein belonging to the immunoglobulin superfamily. CAR is a component of epithelial cell tight junctions and is abundantly expressed in heart, pancreas, the central and peripheral nervous systems, prostate, testis, lung, liver, and intestine.[27] In contrast, several species B and species D adenoviruses bind the transmembrane protein CD46, which is also widely expressed.[28] In addition, a number of other receptors, including CD80 and CD86,[29] sialic acid,[30] and heparan sulfate proteoglycans,[31] have been shown to contribute to attachment and internalization of specific adenovirus types into host cells. It has also been demonstrated that coagulation factor X mediates binding of the adenovirus type 5 hexon protein to hepatocytes, providing a rationale for the hepatic tropism of type 5.[32]

After internalization into endosomes, the virus capsid undergoes conformational changes and is released into the cytoplasm. The virion is then transported by microtubules to the nucleopore, where the adenovirus genome is transferred into the nucleus. Activation of viral transcription leads to expression of early proteins that result in deregulation of the cell cycle and modulation of host antiviral immune responses. These early regulatory proteins are under the control of early region 1A *(E1A)* genes, which in turn control expression from other early genes (*E1B, E2, E3,* and *E4*). The *E3* genes encode several proteins that modulate host immune responses, including inhibition of class I major histocompatibility complex expression and antigen presentation and downregulation of Fas, tumor necrosis factor (TNF), and TNF-related apoptosis-inducing ligand (TRAIL) receptors that lead to inhibition of apoptosis. Within several hours of infection, viral DNA synthesis is initiated, followed by production of viral structural proteins encoded by late genes. New virions are assembled in the nucleus of infected cells and are released by cell lysis.[18]

There is evidence that adenovirus can persist as a latent infection for years after an acute initial infection. Intermittent fecal excretion of species C adenoviruses has been demonstrated to persist for months after an initial acute respiratory infection.[6,33,34] Species D adenoviruses have also been isolated from asymptomatic children.[6,35] Persistent adenovirus secretion into tears has been documented up to 10 years after conjunctivitis.[36] Adenovirus persistence may also be more likely in the setting of immunocompromise. For example, adenoviruses have been identified in the stool of asymptomatic human immunodeficiency virus (HIV)-infected individuals.[37,38] T lymphocytes in tonsils and adenoids are the most likely potential reservoirs of adenovirus because they have been demonstrated to harbor adenovirus DNA for several years in the absence of infectious particles.[39] Although latency has been well described, the mechanisms for this phase of infection are not established.

## EPIDEMIOLOGY

Adenovirus infections are ubiquitous. Most individuals have serologic evidence of prior adenovirus infection by age 10 years, often having experienced infection by several adenovirus types during early childhood.[5] Approximately 50% of all adenovirus infections result in subclinical disease, and most symptomatic infections are mild and self-resolving.[33] Therefore the majority of adenovirus infections remain undocumented, and epidemiologic data are derived from several surveillance studies and investigations of sporadic outbreaks. Epidemiologic studies conducted in the United States in the 1960s and 1970s demonstrated that 5% to 10% of all febrile illnesses in infants and young children are attributable to adenovirus infections, typically involving the respiratory tract and commonly caused by types 1, 2, 3, and 5.[6,33] According to a survey from the United Kingdom, 61% of documented adenovirus infections were present in children younger than 5 years.[40] Based on data from the US National Adenovirus Type Reporting System, begun in 2003, the most commonly reported types of HAdVs during 2003–16 in the United States were HAdV types 1, 2, 3, 4, 7, and 14, which accounted for 85.5% ($n = 1283$) of all types reported.[41] Although types that cause respiratory illness may be transmitted through aerosolized droplets, prolonged secretion after acute infection occurs through the GI tract such that fecal-oral transmission may account for a substantial number of infections in young children.[33] Sporadic outbreaks of pediatric infections have been documented in daycare centers, summer camps, and public swimming pools, among other settings.[41–47]

In contrast, acute adenovirus infections are less common in immunocompetent adults, with the notable exception of military recruits. In this population several epidemics of ARD caused by adenoviruses have been well documented and have led to significant morbidity.[48] Initial studies in military personnel demonstrated that these epidemics were most commonly caused by adenovirus types 4 and 7, leading to the development of a live oral vaccine that was administered to the military until 1999.[49] After discontinuation of this vaccine, outbreaks of adenovirus-related respiratory infections reemerged until a replacement vaccine was reintroduced in 2011, which led to dramatic and sustained decreases in ARD cases among US Army trainees by 2014.[9,50]

In subsequent studies of military personnel investigators have demonstrated that types 3, 4, and 21 are the most common adenovirus infections[10] and that several group B adenoviruses, including types 3, 7, 14, and 21, have caused ARD outbreaks.[10,51,52]

Severe or fatal adenovirus infections in immunocompetent adults are rare, but in 2006 and 2007, several clusters of severe ARD were caused by a virulent strain of adenovirus type 14 that affected military personnel, infants, and immunocompetent adults. The majority of hospitalized patients in these outbreaks were admitted to the intensive care unit, and several patients died, including previously healthy young adults.[7,51] A similarly severe outbreak of adenovirus type 7 causing ARD was reported among a nonmilitary population in Oregon in 2014.[53] In 2008 adenovirus type 55 was recognized as a cause of severe pneumonia among immunocompetent adults in China[54] and has remained an important pathogen in that population.[55] Outbreaks of adenovirus type 55 infection have also been described.[56,57] In addition, adenovirus type 8 was responsible for at least two recent outbreaks of epidemic keratoconjunctivitis in 2016; these outbreaks occurred in the Tibet Autonomous Region of China and the US Virgin Islands.[58,59]

Transmission of adenovirus infections typically occurs through respiratory droplets or the fecal-oral route from individuals with acute infection or asymptomatic viral shedding postinfection. Rare cases of transmission through cervical secretions have been documented in neonates.[8,60] Infections can also be spread by contact with contaminated fomites because adenoviruses can survive for extended periods on environmental surfaces. In one study an isolate responsible for epidemic keratoconjunctivitis (EKC) remained viable for 35 days on an inanimate surface.[61] Nosocomially acquired adenovirus infections have been documented in outbreaks of keratoconjunctivitis[62] and respiratory disease[63] on hospital wards. There is also serologic evidence that adenoviruses can be transmitted in the setting of organ donation.[64]

## CLINICAL SYNDROMES

Most adenovirus infections are self-limited, although fatal infections can occur in immunocompromised hosts, neonates, and occasionally healthy children and adults. Severe disease is also associated with certain adenoviruses, including types 5, 7, 14, and 21. A broad spectrum of clinical adenovirus-associated disease exists, presumably as a result of the diverse types and tissue tropisms of adenoviruses (Table 142.2).

### Respiratory Tract Disease

In children adenoviruses cause approximately 5% of upper respiratory tract infections and 10% of pneumonias.[65] Most commonly, upper respiratory tract disease presents as mild pharyngitis or tracheitis accompanied by coryza. The common types that cause these syndromes are adenovirus 1, 2, 5, and 6, and occasionally 3 and 7. Other systemic manifestations, including fever, malaise, headache, myalgia, and abdominal pain, are common.[66–68] Exudative tonsillitis and cervical adenopathy may be present and can be clinically indistinguishable from group A streptococcal infection.[66] In children younger than 1 year otitis media can also be a common presentation, and adenoviruses have been isolated from middle ear washes in children aged 2 to 12 years with otitis media with effusion.[67,68,69] Adenoviruses have also been associated with a pertussis-like syndrome in cases in which the bacteria were never cultured.[70,71] Several adenovirus types, including 1 to 5, 7, 14, and 21, can cause pneumonia in children and may occasionally result in sequelae such as bronchiectasis. Certain subgroup B adenoviruses (3, 7, 14, and 21) have been associated with

**TABLE 142.2 Clinical Diseases Caused by Adenovirus Infection**

| CLINICAL DISEASE | POPULATIONS AT RISK | CAUSAL ADENOVIRUS TYPES |
|---|---|---|
| Pharyngitis | Infants, children | 1–7 |
| Pharyngoconjunctival fever | Children | 3, 7 |
| Pertussis-like syndrome | Children | 5 |
| Pneumonia | Infants, children | 1–3, 21, 56 |
| | Military recruits | 4, 7, 14 |
| Acute respiratory disease | Military recruits | 3, 4, 7, 14, 21, 55 |
| Conjunctivitis | Children | 1–4, 7 |
| Epidemic keratoconjunctivitis | Adults, children | 8, 11, 19, 37, 53, 54 |
| Gastroenteritis | Infants | 31, 40, 41 |
| | Children | 2, 3, 5 |
| Intussusception | Children | 1, 2, 4, 5 |
| Hemorrhagic cystitis | Children | 7, 11, 21 |
| | HSCT recipients, renal transplant recipients | 34, 35 |
| Meningoencephalitis | Children, immunocompromised hosts | 2, 6, 7, 12, 32 |
| Hepatitis | Pediatric liver transplant recipients | 1–3, 5, 7 |
| Nephritis | Renal transplant recipients | 11, 34, 35 |
| Myocarditis | Children | 7, 21 |
| Urethritis | Adults | 2, 19, 37 |
| Disseminated disease | Neonates, immunocompromised hosts | 1, 2, 5, 11, 31, 34, 35, 40 |

*HSCT,* Hematopoietic stem cell transplant.

severe and complicated pneumonias, particularly in infants. Adenoviruses have also been identified as the cause of community-acquired pneumonia (CAP) in children, with up to 15% of children younger than 5 years with CAP requiring hospitalization, having adenovirus-positive respiratory samples in one study.[72,73] In retrospective studies from South America adenovirus type 7 infection resulted in substantial mortality rates in infants with pneumonia,[74,75] and in an outbreak of adenovirus type 30 infections in neonatal patients in the United States, pneumonia was associated with increased mortality.[76]

Several outbreaks of ARD have been documented in military recruits, most commonly caused by adenovirus types 4, 7, 14, and 21. The clinical syndrome is characterized by fever, sore throat, cough, hoarseness, and rhinorrhea and may progress to involve the lower respiratory tract. Symptoms usually last 3 to 5 days, and on examination pharyngitis, rales, and rhonchi may be present. One study of an outbreak in 2005 showed that pneumonias caused by adenovirus type 14 was associated with higher admission temperature, lower white blood cell count, and lower platelet count than pneumonias not caused by adenovirus type 14, but were not associated with any excess morbidity or mortality.[77] On chest radiographs bilateral patchy ground-glass opacities are consistent with the appearance of viral pneumonia. Rare extrapulmonary complications have been reported, including meningoencephalitis, hepatitis, myocarditis, nephritis, neutropenia, and disseminated intravascular coagulopathy.[78,79,80]

## Ocular Disease

Pharyngoconjunctival fever is a common syndrome consisting of benign follicular conjunctivitis, fever, pharyngitis, and cervical adenitis, commonly caused by adenovirus types 3 and 7. Palpebral and bulbar conjunctivitis may be the sole finding and is typically bilateral. It is a common sporadic illness in children and has also been associated with outbreaks in children's summer camps, swimming pools, and lakes. The illness is usually mild and self-limited.[81]

In contrast, EKC is a more serious illness. Patients present with unilateral or bilateral follicular conjunctivitis, followed by corneal subepithelial infiltrates that are painful and can cause blurry vision. Prominent preauricular lymphadenopathy is common. The incubation period typically lasts 8 to 10 days, and virus can be isolated for up to 9 days after the onset of symptoms.[82] Adenovirus types 8, 19, 37, 53, and 54 have all been documented to cause outbreaks. Although usually self-limited, EKC can take up to 1 month to resolve and is associated with significant patient morbidity. Corneal opacities may persist for several months to years after infection.[83,84] EKC is highly contagious, and outbreaks have been documented in schools, military bases, and hospital wards. Transmission by instruments, eye drops, and skin has been documented in ophthalmic practices, neonatal intensive care units, and the community.[61,62,85–87]

## Gastrointestinal Tract Disease

The detection of adenovirus isolates from the stool of patients with and without clinical disease confounded initial attempts to attribute diarrheal illnesses to adenoviruses. Subclinical infections, confirmed by positive stool cultures and antibody responses, appeared to account for the majority of infections. Furthermore, positive stool cultures without GI symptoms were often observed for weeks to months after respiratory adenoviral disease. Subsequently, the identification of "noncultivable" adenoviruses on electron microscopy examination of symptomatic patients' stools led to the discovery of enteric adenovirus types 40 and 41, which have been closely associated with infantile diarrhea. These viruses can be detected readily by polymerase chain reaction (PCR) and antigen detection assays, and they can be grown in special cell lines. Acute infantile gastroenteritis results in a watery diarrhea that lasts 8 to 12 days on average, accompanied by fever and vomiting.[88,89] In young children approximately 2% to 5% of acute diarrheal illnesses are caused by adenoviruses 40 and 41.[90] Although cases are generally acquired in the community, nosocomial infections have been reported as well. In addition to adenovirus types 40 and 41, types 2, 3, 8, and 31 have been associated with infantile diarrhea in some reports.[91] With the widespread availability of molecular detection techniques, a wide diversity of adenoviruses are now catalogued in many studies of diarrhea in children, including the description of high prevalence of species D in fecal specimens from children in sub-Saharan Africa.[46,47,92] Isolation of adenoviruses from outbreaks of gastroenteritis in adult patients has also been documented, including the initial report of type 52.[93]

Lower-type adenoviruses (1, 2, 5, and 6), but not adenoviruses 40 or 41, are associated with mesenteric adenitis, which can clinically mimic appendicitis and have been shown on occasion to cause intussusception. In these cases adenoviruses have been isolated from stool cultures and lymph nodes. In several studies of children with intussusception, evidence of adenovirus infection ranged from 22% to 61%.[94,95]

## Genitourinary Tract Disease

In children adenoviruses can cause acute hemorrhagic cystitis, which is a benign, self-limited illness. Patients present with gross hematuria lasting 3 days on average, without fever or hemodynamic instability. Microscopic hematuria and dysuria may persist for several more days, but tests for renal function remain normal. Boys are two to three times more commonly affected than girls. In Japan several case series of hemorrhagic cystitis have attributed up to 70% of infections to adenovirus. In the United States only 20% of hemorrhagic cystitis cases can be linked to acute adenovirus infection. Adenovirus types 11 and 21 are most commonly isolated, although adenovirus type 7 has also been detected.[96,97]

Adenovirus types 11, 34, and 35 have caused cases of hemorrhagic cystitis and tubulointerstitial nephritis, reported in renal transplant and stem cell transplant recipients either as isolated syndromes or as part of disseminated disease. In immunocompetent adult males, adenoviruses have been detected in a significant number of cases of

nongonococcal urethritis, and have been associated with adenovirus types 19 and 37.[98,99,100]

### Central Nervous System Disease

Adenoviruses have been associated with sporadic cases of meningitis and meningoencephalitis, either as a primary manifestation or as a complication of systemic or respiratory infection. In rare cases adenovirus has been cultured only from cerebrospinal fluid (CSF) in immunocompetent patients, including from a healthy infant who presented with signs and symptoms of bacterial meningitis,[101,102] and patients undergoing chemotherapy for lymphoma.[103] In one instance adenovirus type 26 was identified by PCR and immunohistochemical staining of a brain biopsy in a patient with medulloblastoma presenting with acute meningoencephalitis.[104] More commonly, meningoencephalitis has been reported as a complication of severe pneumonia, seen primarily with type 7 infection, although also reported with types 1, 6, and 12.[78] Spinal fluid cell counts and chemistries are variable in these cases.

### Other Clinical Syndromes

Myocarditis caused by adenovirus has been described in several case series of acute myocarditis in children, based on detection of virus in myocardial tissue by PCR.[105,106,107,108] In one large study that included neonates and adults, adenovirus PCR of cardiac tissue was positive in 23% of patients with myocarditis, 12% of patients with dilated cardiomyopathy, and in none of the control patients, suggesting that adenovirus may be a common cause of myocarditis.[106]

Rare cases of myositis associated with rhabdomyolysis,[109] arthritis,[110] and pancreatitis[111] caused by adenovirus have been reported. Disseminated adenoviral disease has been best described in pediatric and immunocompromised patients, particularly in neonates, infants, and stem cell transplant recipients. Several adenoviruses, including types 3, 7, 21, and 30, have been isolated in these cases.[112,113]

## INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

Adenoviruses have emerged as important opportunistic pathogens in immunocompromised hosts. Infections can range from asymptomatic shedding of virus to disseminated and potentially life-threatening disease. The majority of clinically significant adenovirus infections occur in hematopoietic stem cell transplant (HSCT) and SOT recipients. One study showed the median time to infection in these populations was 1.6 months posttransplantation, which suggests that these infections were from reactivation disease.[114] The incidence of adenovirus infections in these populations has increased in the past 20 years because of improvements in diagnostic methods, more aggressive conditioning regimens, and the institution of surveillance for adenoviral infection by PCR at some centers.[11,12] These populations are also more likely to have coinfection with more than one adenovirus type.[10]

### Hematopoietic Stem Cell Transplant Recipients

The rates of adenovirus disease in HSCT recipients are difficult to assess because of variations in diagnostics and study inclusion criteria, but mortality rates with adenovirus disease in this population are significant, ranging from 6% to 70% in different case series.[11] In a study of 1050 HSCT recipients, 4.8% of patients were found to shed adenovirus asymptomatically, and 0.9% had invasive disease.[115] Pediatric HSCT recipients have a threefold higher risk of adenovirus infections and are more likely to have severe disease.[116,117] The increased risk of clinical disease in children is likely due to an increased risk of acquiring primary infection, although reactivation of latent infection or reactivation of infection in the transplanted cells may also occur. In addition to younger age, other risk factors for infection include unrelated donor, graft-versus-host disease (GVHD), T-cell depletion of the graft, cord transplants, aggressive immunosuppression, total-body irradiation, and low T-lymphocyte counts after transplantation.[118] Adenovirus is usually detected within the first 100 days posttransplant, with a mean of day 58 and ranging up to day 333 in one study.[119] The presence of adenovirus DNA in blood, a greater degree of immunosuppression, lymphocytopenia, and a rising viral load all increase the risk for serious adenovirus-related clinical disease.[120] In the pediatric HSCT population the most common adenoviral disease is diarrhea or gastroenteritis.[114] Cases of pneumonia, hemorrhagic cystitis, pneumonitis, tubulointerstitial nephritis, hepatitis, cholangiohepatitis, encephalitis, and disseminated disease have been reported as well, with several cases of fatal fulminant hepatic failure due to adenovirus infection reported.[11,121,122] Hemorrhagic cystitis can be severe and prolonged, leading to urethral obstruction by blood clots and occasionally requiring cystectomy for control. There is a wide diversity of adenovirus types that have been identified from samples from stem cell transplant recipients, without one clear type predominating.[123,124]

Surveillance of blood samples by adenovirus PCR to assess risk of infection has become a common practice in some pediatric and adult HSCT centers.[125,126] This practice is based on studies that have shown that adenovirus can be detected in blood 2 or 3 weeks before the development of clinical symptoms.[125] Studies have also shown that increasing viral load measurements have been associated with increased mortality once clinical disease is established. However, the potential use of preemptive therapy in these situations is unclear.[127,128,129,130]

### Solid-Organ Transplant Recipients

In SOT recipients the transplanted organ is typically the primary site of disease.[131] Clinical adenovirus disease may be due to a primary infection or reactivation of latent virus in the transplanted organ because infections are more common in children and in patients with donor-positive/recipient-negative adenovirus status. Severe disease, which may include dissemination, is more common in the pediatric transplant population, particularly liver and lung recipients, and in patients who receive antilymphocyte antibodies.[11] In adults adenovirus infection may be less severe. In one prospective study that included adult liver, heart, and kidney recipients, viremia was documented by PCR in 7% of cases, and more than half of the patients remained asymptomatic and were able to clear the infection spontaneously.[132]

Adenovirus hepatitis has been well described in pediatric liver transplant recipients. In a case series rates of hepatitis ranged from 3% to 10% and frequently led to graft loss and death, with mortality rates up to 53%.[119] Most commonly, hepatitis is caused by adenovirus type 5, but cases caused by adenovirus types 1 and 2 have also been documented. Lung transplant recipients may develop adenovirus pneumonia in the early posttransplant period. One study of adults and children documented a 1.3% prevalence in this population, and subsequent graft failure, death, or bronchiolitis obliterans has been reported.[133,134] Renal transplant recipients can develop acute hemorrhagic cystitis, sometimes complicated by tubulointerstitial nephritis. Adenovirus types 11, 34, and 35 have been detected in these cases. In general, adenovirus infections are less common and less serious in renal transplant recipients, although cases of pneumonia and rare cases of fatal disseminated infections have been reported.[132,135] Adenovirus infections involving the grafted organ have also been reported in cardiac transplant and small bowel transplant recipients.[11] In one study of small bowel recipients, intensive immunosuppressive therapy was associated with progression of infection and systemic adenovirus dissemination.[136] In pediatric cardiac transplant recipients the detection of adenoviruses in myocardial biopsies was associated with reduced graft survival.[137]

### Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome Patients

Most observations regarding adenovirus infections in HIV-positive patients were made before the availability of highly active antiretroviral therapy.[41] In these studies the risk of adenovirus infection was 28% in patients with acquired immunodeficiency syndrome (AIDS) and 17% in patients with CD4 counts greater than 200 cells/μL.[37] Although adenovirus can be isolated frequently from the stool and urine of patients with AIDS, no causative link with diarrhea, hematuria, or other clinical syndromes has been established. Several novel adenovirus types have been detected in specimens from AIDS patients, including many from group D in stool (types 43–51, 58) and respiratory secretions (type 59).[14,138] If symptoms are present, they are usually attributed to other opportunistic infections. Rare cases of fatal hepatic necrosis, fatal pneumonia, encephalitis, nephritis, and systemic infection caused by

adenoviruses have been reported, but adenovirus infections in AIDS patients are an uncommon cause of morbidity or mortality.[139–141]

## DIAGNOSIS

Because most adenovirus infections in immunocompetent patients are mild and self-limited, diagnosis is not routinely pursued. However, establishing a diagnosis may be useful in the setting of outbreaks or for individuals who are immunosuppressed or seriously ill. A confirmed diagnosis can also be useful to help decide whether the use of antiviral medications is warranted or to exclude other treatable infections. Traditional methods of determining adenovirus infection include viral culture, antigen-specific assays, and serologies. Detection by PCR has become widespread because of greater sensitivity and specificity and also rapid turnaround.

With the exception of types 40 and 41, adenoviruses are detectable by routine tissue culture. They grow well in human epithelial cell lines, producing a typical cytopathic effect within 2 to 7 days, although some species D types can take up to 4 weeks to isolate. Viruses may be recovered from nasopharyngeal swabs or aspirates, throat swabs, conjunctival swabs or scrapings, bronchoalveolar lavage fluid, stool or rectal swabs, urine, CSF, and tissue. Viral shedding is detectable in the first 1 to 3 days in patients with pharyngitis, 3 to 5 days in patients with pharyngoconjunctival fever, and up to 2 weeks in patients with keratoconjunctivitis.[142] In immunocompromised patients adenoviruses can be detected in stool intermittently for a more prolonged period of time. For example, adenovirus excretion was detected in the stool of HIV-infected patients for up to 27 months, and prolonged excretion was associated with lower CD4 counts.[37] If culture is not available, direct antigen detection provides rapid diagnosis. The immunofluorescence assay (IFA) is useful for respiratory samples and tissue, and enzyme-linked immunosorbent assay is the test of choice to detect adenoviruses 40 and 41 in stool.[143] The sensitivity of virus detection by IFA of respiratory samples is 40% to 60% lower compared with culture.[144] Viral antigen assays are also less sensitive in immunocompromised patients.[145] Adenovirus infection may also be established by detecting a fourfold or greater rise in adenovirus-specific antibody titers in paired acute and convalescent sera. Detection of adenoviral DNA by PCR has become increasingly attractive for diagnosis, typing of adenovirus, and quantification of virus from a variety of clinical specimens, including fixed tissues, serum, and blood. Primers may be directed against conserved hexon or fiber genes, but more specific typing can be done by a multiplex PCR format, followed by sequencing to detect and identify multiple adenovirus types.[146,147] Real-time PCR has also permitted quantification of virus, which is sometimes used in monitoring viral loads in peripheral blood samples from immunocompromised patients, particularly pediatric HSCT patients. Adenovirus DNA quantification can also be used to measure response to antiviral therapy, and the detection of adenovirus DNA can also establish the diagnosis if found in tissue samples (e.g., endocardial biopsy).[105,129] The specificity of PCR in asymptomatic, immunocompetent adults is high, ranging from 96% to 100% in studies of urine, throat swabs, and peripheral blood.[11,148]

Typing of viral isolates is not routine but can be determined in a reference virology laboratory. Traditionally, typing has been determined by hemagglutination patterns and serum neutralization assays against a panel of type-specific sera. Recently, whole-genome sequencing and phylogenetic analyses of adenoviruses have shown that serum neutralization may be misleading, because neutralization is primarily directed against small portions of viral capsid proteins. In 2011 new criteria for characterizing and typing novel human adenoviruses were proposed and include analysis of the complete genome sequence, with continued use of serum neutralization as an additional criteria.[24]

When obtained, tissue should be sent for culture and pathologic examination. Histopathologic findings in the lung include diffuse interstitial pneumonitis, necrotizing bronchitis, bronchiolitis, and pneumonia with mononuclear cell infiltration and hyaline membrane formation.[149] Early postinfection, infected cells may display small eosinophilic inclusions. During late infection basophilic intranuclear inclusions surrounded by a thin, clear halo emerge and eventually enlarge to obscure the nuclear membrane. This produces "smudge cells," which are characteristic of adenovirus infections (Fig. 142.3). In contrast to cytomegalovirus, there are no intracytoplasmic inclusions or multinucleated giant cells. Further study by electron microscopy, adenovirus-specific immunohistochemical assays, and in situ DNA hybridization can be performed to make the diagnosis.

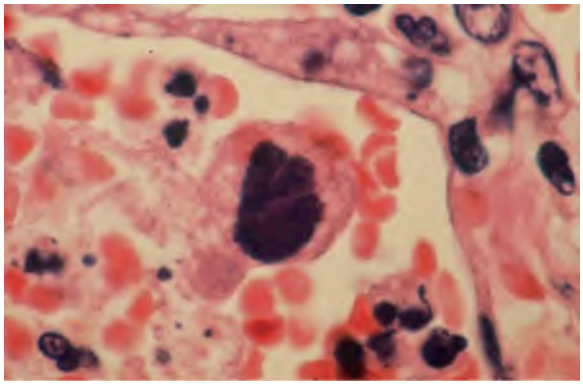

**FIG. 142.3 Lung biopsy specimen from a patient with adenovirus pneumonia showing a characteristic "smudge cell" (hematoxylin and eosin stain, ×400).** *(Courtesy Franz C. Lichtenberg, MD, Department of Pathology, Brigham and Women's Hospital, Boston, MA.)*

## THERAPY

There are currently no approved antiviral agents for the treatment of adenovirus infections. Clearance of adenovirus infection in HSCT and SOT patients is typically associated with immune reconstitution, particularly with improved absolute lymphocyte counts and CD4 T-cell counts.[150,151] There are few prospective, controlled trials of antiviral drugs for adenovirus infections, and thus clinical experience is mostly limited to retrospective case series and case reports, primarily in immunosuppressed patients. Cidofovir has good in vitro activity against adenovirus and has been reported to be useful in certain animal models of ocular adenovirus infections.[152] However, a large, multicenter trial to evaluate the potential efficacy of topical cidofovir for EKC was discontinued because of toxicity.[153] In several case series and case reports involving pediatric and adult HSCT recipients, with a variety of clinical adenovirus syndromes, treatment with cidofovir appeared to be associated with clinical improvement in a subset of patients, although fatalities still occurred and significant nephrotoxicity was noted.[117,154,155,156] Moreover, it was not clear if the clinical improvement in these individuals was due to the drug. Studies are currently evaluating an oral liposomal formulation of cidofovir (CMX001, brincidofovir) for severe adenovirus infection in immunocompromised patients[157] (also see Chapter 46). Retrospective case series have suggested that brincidofovir is well tolerated and that treatment can lead to decreases in adenovirus loads.[158,159] A phase II study of brincidofovir in pediatric and adult HSCT recipients with asymptomatic adenovirus viremia demonstrated that brincidofovir resulted in rapid and sustained virologic responses in patients with high viremia at baseline.[160] However, the drug was also associated with GI toxicity in some patients and more frequent incidence of acute GVHD. A phase III multicenter study of brincidofovir comparing treatment of early versus late adenovirus infection in HSCT or SOTs will hopefully clarify the safety and efficacy of brincidofovir; the study has been completed, but the results are not yet published.[161] Ribavirin use has been reported in several cases of adenovirus infection in HSCT recipients, but results in case series have been mixed[162,163] and may be explained in part by the observation that in vitro activity of ribavirin appears restricted to group C types.[164] Vidarabine and ganciclovir are reported to possess in vitro activity against adenovirus, but there are scant clinical data for these agents.[165] In some centers preemptive therapy has been proposed to treat patients who are at risk for adenovirus infection. In a study of 58 pediatric HSCT recipients in which patients were prospectively screened weekly for evidence of infection and then treated with cidofovir, symptoms and viremia resolved in the majority of recipients.[154] It is clear that larger prospective studies are needed to determine if any of these antiviral agents have clinical efficacy and whether their use is warranted in particular clinical settings.

Immunotherapy by adoptive T-cell therapy is being pursued by some groups but remains an investigative approach. The idea is that the restoration of virus-specific immunity in immunocompromised individuals could be more effective at suppressing adenovirus than antiviral drugs, while being less toxic and less likely to induce resistance. Small studies have shown that infusion of adenovirus-specific T cells into HSCT patients led to the induction of effective T-cell responses, reductions in adenoviral load, and instances of clinical improvement of adenoviral-related disease in some individuals.[166,167,168–170] These results were followed up by an open-label clinical trial of the safety and efficacy of ex vivo adoptive T-cell transfer with hexon-specific T cells in 30 patients with adenovirus disease or viremia. In this study immunotherapy led to in vivo antiviral immunity for up to 6 months with viral control, resulting in complete clearance of viremia in 86% of patients with antigen-specific T-cell responses, and the suggestion of a mortality benefit in responders.[171] Intravenous immune globulin has also been used in immunocompromised patients with mixed results.[156,172,173]

## PREVENTION

Because of the morbidity seen with respiratory adenovirus infections in military recruits, successful live oral vaccines for types 4 and 7 were developed and administered in the military starting in 1971. The vaccine was packaged in enteric capsules that ensured that replication would occur in the GI tract and not the airways, resulting in subclinical infection and good neutralizing antibody responses. The sole manufacturer discontinued vaccine production in 1996, and vaccination stopped in 1999 when the supply was exhausted. Subsequently, ARD recurred in military recruits at rates similar to those of the prevaccination era, with several outbreaks affecting up to 80% of recruits, resulting in hospitalization rates ranging from 11% to 20% and producing occasional fatalities.[52,174] After a decade of development, the vaccine was restored to use in the military in 2011, and rates of adenovirus disease burden have fallen by more than 100-fold in military trainees, without an increase in diseases associated with adenoviruses not present in the vaccine.[175,176]

## ADENOVIRUSES AS VECTORS FOR GENE THERAPY AND VACCINATION

There has been intense investigation during the past two decades into the capacity of adenoviruses to serve as a vector platform for delivery of genes for both gene therapy and vaccination. Typically, adenoviruses are rendered replication incompetent by deletion of the *E1* gene, which allows the insertion of a transgene expression cassette that encodes a gene of interest. Scores of human clinical trials using adenovirus vectors have been conducted, and others are currently in progress or are planned. Adenovirus vectors have been well studied and have several advantages over other available vectors, including their ability to be produced at high titers, to infect several cell types, including both dividing and nondividing cells, and to accommodate gene inserts stably.

Applications for gene therapy have primarily focused on delivery of a functional gene to replace a dysfunctional or absent gene product for diseases such as cystic fibrosis, ornithine transcarbamylase deficiency, hemophilia, and bilirubin uridine diphosphate glucuronosyl transferase deficiency. Although promising in several animal models, early human gene therapy studies have proven disappointing because of inefficient gene delivery.[177] Initial studies also demonstrated that adenovirus vectors at high doses elicit early innate immune responses, including production of proinflammatory cytokines that can lead to systemic toxicity and resulted in the death of a volunteer in a clinical trial.[178,179] Vector-specific immune responses also develop rapidly and limit the utility of repeat vector administration. Several strategies to minimize vector-specific immunity have been developed, including using different human adenovirus types, nonhuman adenoviruses, and structurally modified adenoviruses to create novel vectors.

Adenovirus vectors are also being developed as vaccines for both infectious diseases and cancer, primarily because of their ability to elicit robust cellular immune responses against encoded transgenes. These vectors contain genes encoding pathogen-specific antigens and elicit robust $CD8^+$ T-lymphocyte responses and polyfunctional antibody responses.[180] Adenovirus vector–based vaccine candidates are currently being explored for a variety of infectious diseases, including malaria, herpes simplex virus, tuberculosis, Ebola, Zika, and HIV-1 infections.[181–183] Clinical trials for adenovirus vectors are also being conducted in the tumor vaccine field. Strategies include directly delivering genes that control cell growth, apoptosis, and angiogenesis, or genes that express cytokines to induce antitumor responses. Another approach has been to modify adenovirus vectors to replicate and induce lysis in tumor cells but not normal cells. Approximately a dozen clinical trials are currently evaluating oncolytic adenoviruses for the treatment of ovarian, prostate, pancreatic, and other solid tumors, although this concept has yet to demonstrate clinical efficacy.[184,185]

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*The complete reference list is available online at Expert Consult.*


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# 143 Papillomaviruses

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## SHORT VIEW SUMMARY

### Definition

- Human papillomavirus (HPV) infects the squamous stratified epithelia of the body and causes tumors that can be benign (warts, condylomas, papillomas) or malignant (squamous cell carcinomas, uterine cervical adenocarcinoma).
- They cause two main groups of diseases: (1) cutaneous (hand, foot, flat) warts and (2) lesions of the mucosal or genital surfaces, such as genital warts; laryngeal papillomas; and cancers of the cervix, vagina, vulva, anus, penis, oropharynx, and their respective precursor lesions, called intraepithelial neoplasias (dysplasias).

### Epidemiology

- Cutaneous warts are predominantly a disease of school-aged children. They are acquired from close contacts, predominantly in the family environment.
- Genital (or mucosal) HPV infections are mostly sexually transmitted, and their incidence peaks in late adolescence and early adulthood.
- Most of the sexually active population will have been exposed to genital HPV in a lifetime.
- Genital HPV infections in males and females are easily acquired, but most also disappear quickly. Persistence is a risk factor for the development of cancer.

### Microbiology

- HPVs are small, nonenveloped DNA viruses, classified according to the nucleotide sequence of the gene coding for the major capsid protein. These viruses are not routinely cultivable.
- At least 210 types have been identified, but only a small number carry the bulk of the health burden.
- HPV types 1, 2, and 4 are the most common types found in cutaneous warts.
- HPV types 6 and 11 account for most genital warts.
- HPV types 16 and 18 cause the great majority of cancers of the anogenital tract and oropharynx and are defined as high-risk oncogenic.
- The more severe the grade of intraepithelial neoplasia, the more prevalent are high-risk oncogenic HPVs in the lesion.

### Diagnosis

- The diagnosis of cutaneous warts and of genital warts is typically clinical. A biopsy is indicated when the diagnosis is in doubt or a malignancy or its precursor is a consideration.
- For the screening of cervical cancer, cytology (Pap smear) is the primary diagnostic approach.
- HPV DNA testing supplements screening cytology.

### Therapy

- Many therapeutic modalities exist for the treatment of HPV-induced lesions, none of them fully satisfactory. They can be divided into medical and physical approaches.
- The chemical methods include salicylic acid solutions for cutaneous warts or podofilox or imiquimod for genital warts.
- The physical methods include cryotherapy, cold-blade excision, electrosurgery, and laser therapy, and they can be applied to most lesions.

### Prevention

- Male condoms have some effectiveness in protecting against genital infections.
- Pap smears are essential for the prevention of cervical cancer.
- Vaccination is very effective and safe in preventing genital warts and intraepithelial neoplasias of the cervix, vagina, vulva, and the anus in males and females, and cervical cancer
- A 9-valent vaccine (Gardasil 9) is available in the United States. This vaccine is not infectious and protects against HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58. It has now replaced an earlier quadrivalent vaccine (Gardasil) that covered only the first four of these genotypes. It is administered intramuscularly in a two- or three-dose schedule. Cervarix, a bivalent vaccine covering only HPV-16 and HPV-18 is still available in some parts of the world.

Papillomaviruses have been detected in a variety of higher vertebrates. *Human papillomaviruses* (HPVs) are widespread throughout the population, produce epithelial tumors of the skin and mucous membranes, and have been closely associated with genital tract malignant diseases. HPVs are strictly species specific, and cross-species infections do not occur even in experimental conditions. The infectious nature of human warts was initially seen in the late 19th century when human wart extracts were shown to produce warts with injection into humans. Ciuffo[1] suggested that the infectious agent of warts was a virus, after he was able to transmit the infection through cell-free filtrates in 1907. Despite these early observations, HPVs have not been studied with standard virologic techniques because they have not been propagated successfully in tissue culture or in standard laboratory animals. For this reason, much of our knowledge of the biology of HPVs and the diseases with which they are associated has depended on the use of molecular biologic techniques and, more recently, of organotypic cultures and complex animal models. These techniques have led to an understanding of the genomic organization of these viruses, the functions of different viral genes, and the multiplicity of HPV types. Detailed reviews of these subjects are available.[2–6]

## VIROLOGY

Papillomaviruses constitute the *Papillomavirus* genus of the Papillomaviridae family. They are nonenveloped viruses that are 55 nm in diameter

and have an icosahedral capsid composed of 72 capsomeres that enclose a double-stranded circular DNA genome. Virion particles contain at least two capsid proteins. The major capsid protein constitutes 80% of the virion by weight and has a molecular weight of about 56,000 daltons. The minor capsid protein has a molecular weight of approximately 76,000 daltons.

The HPV genome consists of approximately 7900 base pairs. All putative coding sequences (open reading frames [ORFs]) are arranged on one DNA strand, and all papillomaviruses share the same genomic organization.[6–13] Specific protein products are derived from these ORFs. However, analyses of viral messenger RNA (mRNA) transcripts suggest that most viral proteins derive from splicing of more than one ORF-specific mRNA. The genome is divided functionally into three regions. A noncoding upstream regulatory region contributes to the control of DNA replication and transcription of eight to nine ORFs that are divided into early (E1–E7) and late (L1 and L2) regions.[6–13] E1 is involved in viral plasmid replication.[10–12] The E2 product is an important modulator of viral transcription and also plays a role in viral replication.[10–12] E4 proteins form filamentous cytoplasmic networks and share the same cellular distribution as cytokeratin intermediate filaments, with which they may interact.[2,3,12] The E5 protein is located in the cellular membrane and prevents the acidification of endosomes.[12] It stimulates the transforming activity of the epidermal growth factor receptor and contributes to the oncogenicity of HPV.[3,9,11–13] The gene products of E6 and E7 of oncogenic HPV types have major transforming properties through the binding of various cellular factors and key tumor suppressor proteins.[1–3,7,9,11–13] The E6 protein binds to the p53 tumor suppressor gene product and abrogates its activity by accelerating its degradation. The E7 protein also binds to a tumor suppressor gene product, the retinoblastoma protein, and to related proteins, thus inhibiting their functions. Both E6 and E7 proteins can impede apoptosis. The L1 and L2 ORFs encode the major and minor capsid proteins, respectively.[1–3,7,13]

Although the genomes of several papillomaviruses can transform certain cell lines in tissue culture, only recently have both replication and propagation of HPV been possible in vitro, with use of organotypic culture systems.[8,14] In addition, HPV types 6, 11, 16, 40, and 59 have been propagated successfully in human skin grafted in the (nude) mouse or the mouse with severe combined immunodeficiency (SCID).[15] HPV-infected grafts recovered from these animals can maintain viral particle production in vitro.

Virions of most HPV types cannot be purified from naturally occurring lesions in significant quantities, and well-characterized type-specific antigens have not been available until recently.[16] Therefore types are determined according to the degree of nucleic acid sequence homology rather than with serologic techniques. Distinct HPV types share less than 90% of DNA sequences in the L1 ORF, subtypes share between 90% and 95%, and variants between 95% and 98%.[7,17] According to common nomenclature, HPVs belong to five genera of the 49 in the Papillomaviridae family: alpha, beta, gamma, mu, and nu. A genus may be further divided into species. For example, the alphapapillomavirus HPV-16 is the representative type of species 9, which also includes types 31, 33, 35, 52, and 67. At least 210 HPV types have now been characterized, and many others have been recognized. HPVs are host specific, and each type is, to a large extent, associated with a distinct histopathologic process (Table 143.1).

A broadly cross-reactive genus-specific antigenic determinant, located in the middle of the major capsid protein,[18] can be prepared with denaturation of viral particles, typically from bovine papillomavirus, with detergents and reducing agents. Antisera prepared against this papillomavirus common antigen have been used in the immunocytochemical diagnosis of HPV infections (see "Diagnosis").[19] The antigenic characteristics of native viral particles can also be studied with the use of virus-like particles (VLPs). These are obtained with the expression in eukaryotic systems of the L1 or L1 and L2 ORFs (see "Prevention").[14] A close correlation generally exists between genotype and serotype.[16]

**TABLE 143.1 Human Papillomavirus Types and Their Disease Association**

| DISEASE | HPV TYPES[a] FREQUENT ASSOCIATION | HPV TYPES[a] LESS FREQUENT ASSOCIATION |
|---|---|---|
| Plantar warts | 1, 2, 27 | 4, 26,[b] 28, 29, 41,[c] 57, 63, 65, 77,[c] 117,[b] 125,128, 129, 130, 131, 132, 133, 148, 149, 179, 184 |
| Common warts | 1, 2, 4, 27 | |
| Common warts of meat, poultry, and fish handlers | 2, 7 | 1, 3, 4, 10, 28 |
| Flat and intermediate warts | 3, 10 | 27,[b] 28, 38, 41,[c] 49,[b] 75, 76, 126[b] |
| Epidermodysplasia verruciformis | 5,[c] 8,[c] 9, 12, 14,[c] 15, 17[c] | 19, 20,[d] 21, 22, 23, 24, 25, 36, 37, 38, 47,[c] 49, 50, 75, 93 |
| Condylomata acuminata | 6, 11 | 16,[c] 18,[c] 26,[c] 31,[c] 33,[c] 35,[c] 40, 42, 43, 44, 45,[c] 51,[c] 52,[c] 53,[c] 54, 55, 56,[c] 58,[c] 59,[c] 66, 68,[c] 70, 153, 175, 178, 180, 200, 201, 202 |
| Intraepithelial neoplasia, unspecified | | 26,[c] 30,[c] 34, 39,[c] 40, 53,[c] 57, 59,[c] 61, 62, 67,[c] 68,[c] 69, 71, 81, 83 |
| Low grade | 6, 11 | 16,[c] 18,[c] 31,[c] 33,[c] 35,[c] 42, 43, 44,[d] 45,[c] 51,[c] 52,[c] 54, 61, 70, 72, 74[b] |
| High grade | 16,[c] 18[c] | 6, 11, 31,[c] 33,[c] 34,[b] 35,[c] 39,[c] 42, 44, 45,[c] 51,[c] 52,[c] 56,[c] 58,[c] 66,[c] 67[c] |
| Cervical carcinoma | 16,[c] 18[c] | 26,[c] 31,[c] 33,[c] 35,[c] 39,[c] 45,[c] 51,[c] 52,[c] 56,[c] 58,[c] 59,[c] 66,[c] 67,[c] 68,[c] 73,[b,c] 82[c] |
| Recurrent respiratory papillomatosis | 6, 11 | 16,[c] 18,[c] 31,[c] 33,[c] 35,[c] 39[c] |
| Focal epithelial hyperplasia of Heck | 13, 32 | 18,[c] 33,[c] 45[c] |
| Conjunctival papillomas and carcinomas | 6, 11, 16[c] | |
| Other cutaneous lesions[e] | | 26,[b,c]36, 37, 38,[c] 41,[c] 48,[b,c] 60, 72,[b] 88, 92, 93, 94, 95, 96, 107, 110, 111, 155, 174, 197[c] |
| Other genital lesions | | 26,[b,c] 30,[c] 84,[c] 85, 86,[c] 87, 89, 90, 91, 97, 101, 102, 103, 106, 175, 180, 199 |
| Healthy cutaneous or mucosal tissue | | 80, 114, 115, 116, 118, 119, 120, 121, 122, 123, 124, 127, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 150, 151, 156, 157, 158, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 199, 205, 209 |

[a]The distinction between frequent and less frequent association is arbitrary in many instances. Large descriptive statistics of HPV type distribution by disease are not available for most HPV types. Moreover, many HPV types have been looked for or identified only once.
[b]Types first recovered from patients with immunosuppression or immunodeficiency.
[c]Types with high malignant potential or isolated in only one or a few lesions that were malignant.
[d]HPV-46 was found to be HPV-20, HPV-64 is a variant of HPV-34, and HPV-55 is HPV-44.
[e]Includes epidermoid cysts, keratoacanthoma, laryngeal carcinoma, and malignant melanoma.
*HPV,* Human papilloma virus.
*Information on HPV DNA sequences and types is available at http://pave.niaid.nih.gov/#home and http://www.nordicehealth.se/hpvcenter/reference_clones/. Note that the sequence information on some of the newer genotypes is not yet available.*

## EPIDEMIOLOGY

### Incidence and Prevalence

Although clinical HPV infections are the most recognizable and most important for the patient and practitioner, subclinical and asymptomatic

latent infections are probably most common, and past HPV infections represent an even larger group.[20–22] The study of these different types of infection poses different technical problems, and their respective interrelated epidemiologies are not equally well understood.

As Table 143.1 illustrates, HPV infections can also be divided according to predominant anatomic location of the lesions they cause. Thus the genital or mucosal infections are recognized as distinct from the nongenital infections, which include the cutaneous infections. The genotypes associated with asymptomatic infections are less restricted in their anatomic distribution.[23–25]

Three types of cutaneous HPV infections are widespread throughout the general population.[26] Common warts, which represent up to 71% of all cutaneous warts, occur frequently among school-aged children, with prevalence rates of 4% to 20%.[27,28] Although less common (34% of cutaneous warts), plantar warts are observed frequently among adolescents and young adults. Juvenile or flat warts are the least common of the three types (4%) and occur predominantly in children. Other groups at high risk for the development of cutaneous warts include butchers, meat packers, and fish handlers.[29] Epidermodysplasia verruciformis is a rare, typically autosomal recessive condition characterized by the appearance early in life of disseminated cutaneous warts and frequent malignant transformation.[30]

Large surveys in the United States have shown that the prevalence of any and high-risk genital HPV in 18- to 59-year-olds was 45.2% and 25.1% in males, and 39.9% and 20.4% in females, respectively.[31] Accordingly, 50 million Americans are likely infected and contagious. Peak prevalence was reached in the age-class 25 to 29 years in males and essentially remained unchanged in the older groups.[32] In females the peak prevalence was in the 20- to 24-years age-group, and declined by about one-fifth in the older cohorts.[33] Most of the sexually active population is likely to be infected in a lifetime.[20]

The prevalence rate of condyloma acuminatum (plural, condylomata acuminata) or anogenital warts (venereal warts) in the general population ranges from 0.2% to 5%, based on genital examination.[34] In a US survey of 18- to 59-year-olds, 4.0% of males and 7.2% of females reported having had genital warts.[34] The incidence of the disease has risen. The annual number of initial visits to physicians' offices for genital warts doubled between 2000 and 2014, from 220,000 to 465,000.[35] It has since remained stable.[36] However, with the introduction of a quadrivalent HPV vaccine in 2006, the prevalence of genital warts has dropped between 2006 and 2010 from 2.9 to 1.8 per 100 person-years in females aged 15 to 19 years, the cohort most likely to have been vaccinated.[36] HPV infection of the cervix gives rise to the most common cause of squamous cell abnormalities on Papanicolaou (Pap) smears and are found in two-thirds of 1000 females aged 15 to 39 years.[36] The incidence rate of recurrent respiratory papillomatosis, which is primarily a disease of the larynx, is estimated to be 4.3 per 100,000/year for the juvenile-onset form of the disease (peak prevalence age, 7 years) and 1.8 per 100,000/year for the adult-onset form (bimodal peak prevalence ages, 35 and 64 years).[37,38] Prevalence rates are about twofold to ninefold greater.[37] The prevalence of oral HPV infections is 7.5%, but that of associated lesions is 0.5%, although higher in human immunodeficiency virus (HIV)-infected subjects, particularly on highly active antiretroviral therapy.[39,40]

## Transmission

Close personal contact, especially within the family and school class, is likely to be important for the transmission of most cutaneous warts.[29,41] Minor trauma at the site of inoculation may also be important, as suggested by the high frequency of disease among meat handlers.[29]

Evidence that anogenital warts are sexually transmitted includes the observations that the age of onset is similar to that in other sexually transmitted diseases (STDs) and that the disease develops in approximately two-thirds of sexual contacts of patients with anogenital warts.[42,43] In addition, patients with anogenital warts often have other concomitant STDs or a history of such infections. Also, as outlined in Table 143.1, particular HPV types are associated with these lesions. These types are rarely found in lesions at other sites. Finally, a large number of lifetime, present, or recent sexual partners; the frequency of sex or other intimate skin-to-skin contact; and the sexual histories or behavior of sex partners are risk factors of genital HPV transmission, whereas circumcision in some studies has been found to be protective, as with HIV and herpes simplex virus (HSV).[20,44–46] Despite these observations, in adults routes of transmission not involving the penis are possible, but their relative importance, probably small, remains unclear.[47] Young children may acquire genital warts from hand contact with nongenital lesions.[48] Approximately one-fifth of prepubertal children with condyloma acuminatum have HPV type 1 or 2 in the lesions.[49–51] Conversely, HPV-6 DNA has been identified in cutaneous warts of family contacts of children with anogenital warts.[50]

In adults estimates of the rate of HPV transmission do vary.[52,53] In one study these rates (expressed as number of events per 100 person-months) were 3.5 from penis to cervix and 4.0 from cervix to penis.[54]

Recurrent respiratory papillomatosis in young children is thought to be acquired via passage through an infected birth canal or through the placenta.[37] This hypothesis is based on the observations that HPV DNA is frequently recovered from placentas or cervicovaginal lavages of pregnant women, as well as in neonates.[55] Furthermore, similar HPV types are associated with both respiratory papillomatosis and anogenital warts and that a large percentage of the mothers of these children have a history of genital tract HPV disease.[37] In addition, neonates are more likely to harbor HPV DNA in the oral cavity if the cervix of the mother contains HPV DNA.[37] Many children with recurrent respiratory papillomatosis are first born babies who were delivered vaginally to young (often teenage) mothers. Although the median age of onset of recurrent respiratory papillomatosis is 3 years, cases have been documented at birth, even after cesarean section.[37] This observation suggests that the disease may be acquired in utero, probably via ascending infection from the mother's genital tract. The role of cesarean section, if any, in prevention of transmission is unknown, and the procedure is not recommended for that purpose.[37] Family members and others with close personal contact with these patients are not at risk for developing the disease. In the adult-onset form recurrent respiratory papillomatosis is associated with a higher-than-expected number of lifetime sexual partners and with oral-genital contact.[37]

The role of fomites in the transmission of HPV infection is uncertain.[47] However, nosocomial transmission appears possible because infectious virus can be recovered from the fumes released from lesions during treatment with a carbon dioxide ($CO_2$) laser or electrocoagulation.[56] In addition, HPVs are resistant to heat, and use of an autoclave is probably necessary for sterilization of contaminated instruments.[57,58]

## Association Between Human Papillomavirus and Malignant Diseases

The oncogenic potential of animal papillomaviruses was shown many years ago.[59] Observations of patients with epidermodysplasia verruciformis provided the initial evidence that suggested that HPVs might also be carcinogenic. In these patients characteristic skin lesions induced by specific HPV types frequently undergo malignant transformation, particularly when they occur in sun-exposed areas.[30] Most research investigating the oncogenic potential of HPVs has focused on genital tract malignant diseases.[7]

The low prevalence of cancer of the uterine cervix among Catholic nuns,[60] the direct association of risk with number of sexual partners, and the increased risk of malignant disease that is associated with a male sexual partner whose previous consort had cervical cancer have been observations consistent with a sexually transmitted agent playing a role in the pathogenesis of cervical cancer.[3,7,12,21,61–63] Among several agents, HSV-2 was once strongly suspected. However, over the past 40 years a large and coherent body of biologic and epidemiologic observations has shown that HPV infection is the necessary, if not sufficient, cause of cervical cancer.[64] This evidence can be summarized as follows:

1. The association between those HPV types called high-risk oncogenic (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66, as classified by the International Agency for Research on Cancer) and cervical cancer is strong, with odds ratios (ORs) that range from 50 to 100.[7] For the most oncogenic of these viruses, HPV-16 for squamous cell carcinoma (SCC) and HPV-18 for

adenocarcinoma, the ORs range from 100 to 900. In a worldwide survey HPV DNA was found in 99.7% of cervical cancer samples.[65]

2. The association has been consistent through many studies done in different countries and populations.
3. The association has been specific, so that among the approximately 40 HPV types associated with the genitalia, or more broadly the mucosal surfaces, only a subset of the types (at least 15) are oncogenic for the cervix. In addition, the same HPV types, with an even greater predominance of HPV-16 or HPV-18, are found in other SCCs. The fraction of SCCs attributable to HPV is 69% for the vulva, 75% for the vagina, and 63% for the penis.[66] The figures are even higher if only warty and basaloid histologic variants of these tumors are considered.[67] HPV is found in 91% of anal SCCs and, for the period 2005–09, in 72% of oropharyngeal SCCs.[66,68] Before 2000, only 40.5% of oropharyngeal SCCs were associated with HPV.[68] HPV-associated oropharyngeal cancer is now more common than cervical cancer in the United States.[69] This sharp increase has not been seen with the nonoropharyngeal head and neck cancers; the percentage attributable to HPV has remained at 22%.[68]

   Largely on the basis of molecular epidemiologic studies, mucosal high-risk HPVs have been found in cancers of the esophagus, lung, and breast but also of the colon, urothelium, prostate, the ovary, and endometrium, thus raising a possible causal role in these tumors. However, the nature, consistency, and strength of these associations are controversial.[70–77] HPV-16 has been found in some SCCs of the conjunctiva and of the nail bed.

   The beta HPV types found in the SCCs of patients with epidermodysplasia verruciformis have also been found in about a third of keratinocyte carcinomas (SCCs and basal cell carcinomas) in immunocompetent hosts and in up to 80% of immunosuppressed hosts. There is growing evidence that this association is causal, but through mechanisms analogous yet distinct from those associated with the high-risk mucosal, alpha HPVs.[78,79]
4. The development of cervical abnormalities and cancer is preceded by HPV infection. Most HPV infections are transient and last a mean of 13.5 months for high-risk HPVs and 4.8 months for low-risk types. However, between 15% and 30% of women with normal cervical cytology but high-risk HPV infection have cervical intraepithelial neoplasia (CIN) grades 2 or 3 develop in the following 4 years. Conversely, CIN 2 or 3 is unlikely to develop in women with milder cytologic squamous abnormalities who are negative for high-risk HPV. Although clearance of HPV DNA appears to precede clearance of cervical lesions, persistence of HPV DNA after treatment for CIN 2 or 3 is a predictor of relapse.

   The temporal association between HPV and cervical premalignant lesions has proven to be useful for prevention strategies. Hence HPV testing is more sensitive than repeated cervical cytology in identification of women with atypical squamous cells of unknown significance (ASC-US) than those with CIN 2 or 3.

   The number of sexual partners, the age of first sexual intercourse, and the sexual behavior of the husband are risk factors for HPV infections and also for cervical cancer, which occurs later in life. This temporal sequence is consistent with a causal link between infection and cancer.
5. In some studies a direct association is found between viral load and the risk of cancer, which is consistent with a biologic gradient.
6. Several lines of biologic evidence support an oncogenic role for HPV. Virtually all neoplastic cells in cervical cancer tissue contain HPV DNA, including metastases. The *E6* and *E7* genes are expressed at higher levels in neoplasms than in benign lesions. When the *E6* or *E7* genes of high-risk HPV types are introduced in normal cells, they cause malignant transformation in cell culture. Transgenic animals carrying these genes develop SCCs. The role of E6 and E7 proteins is further discussed in the "Pathogenesis" section.
7. Papillomaviruses can cause cancer in animal experimental models, such as the cottontail rabbit papillomavirus in the domestic rabbit and bovine papillomaviruses in the cow. Moreover, human neonatal foreskin grafts infected with HPV-16 and placed in SCID mice develop intraepithelial neoplasias.[80]
8. Other alternative risk factors for cervical cancer, such as the use of oral contraceptives, high parity, tobacco smoking, nutrition (vitamins C and E, carotenoids, xanthophylls), immunosuppression, prior HSV-2 or *Chlamydia trachomatis* infection, have not reached the strength and coherence of the evidence gathered for HPV. Their contribution may be only secondary to the primary role played by HPV infection.
9. Finally, the clinical trials of the HPV vaccine (see "Prevention") have amply shown that immunization against HPV types 6, 11, 16, and 18 confers protection not only against subsequent infection but also against disease (warts and intraepithelial neoplasias of all grades) caused by the homologous genotype in the cervix, vagina, and vulva and in the male external genitalia and anus.

## PATHOGENESIS

The pathogenesis of HPV disease has been reviewed by several authors.[1–4,6,7,9–12] The incubation period was established experimentally with inoculation of human subjects with extracts of cutaneous warts.[1,81] Most often, warts developed within 3 to 4 months, although lesions occasionally grew as early as 6 weeks or as long as 2 years after inoculation. A similar incubation period was observed for genital warts among wives of American soldiers returning from the Korean War.[82] All types of squamous epithelium may be infected by HPV, but with the exception of the cervical glandular epithelium, other tissues appear to be resistant to productive infection. Gross histologic appearances of individual lesions vary with the site of infection and the virus type. Fig. 143.1 is a schematic diagram of a typical exophytic cutaneous wart.

The virus replicative cycle is tightly dependent on epithelial differentiation. It begins with the entry of particles into the stratum germinativum (basale) because viral DNA is detected in the nuclei of the basal cells.[2,3,11,12,83] It requires a breach of the integrity of the epithelium so that the viral particle can bind the heparan proteoglycans present on the basement membrane and basal cell. It initiates a process that includes a modification of the capsid conformation and proteins that allows entry by endocytosis facilitated by several possible candidate host molecules.[83] As the basal cells differentiate and progress to the surface of the epithelium, HPV DNA replicates and is transcribed, and viral particles are assembled in the nucleus. Ultimately, complete virions are released, probably still tightly associated with the remnants of the shed dead keratinocyte shell.[11,84] In a wart or condyloma, viral replication is associated with excessive proliferation of all of the epidermal layers except the basal layer. This process produces acanthosis, parakeratosis, and hyperkeratosis. A deepening of the rete ridges, where normally present, produces the typical papillomatous cytoarchitecture. Some infected cells undergo the characteristic transformation of koilocytosis. With histology, koilocytes (from the Greek *koilos*, "cavity") are large, usually polygonal, squamous cells with a shrunken nucleus lodged inside a large cytoplasmic vacuole. Cytoplasmic keratohyalin inclusion bodies may also be observed. Excessive proliferation of the basal-like cells (basaloid proliferation) with a high nuclear/cytoplasmic ratio, accompanied by a high number of mitoses (some abnormal [dyskaryosis]), is a feature of incipient and malignant HPV disease.

Normal-appearing epithelium may contain HPV DNA,[85,86] and the presence of residual DNA after the treatment of warts may lead to recurrent disease. In benign lesions caused by HPV, viral DNA is located extrachromosomally in the nuclei of infected cells. However, when HPV DNA is detected in high-grade intraepithelial neoplasias and cancers, it is generally integrated.[2–4,8,9,12] Integration of HPV DNA may occur at preferential sites in host cell chromosomes,[3] and it specifically disrupts the E2 ORF. Interruption of E2 probably plays a role in the pathogenesis of malignant disease because expression of this ORF normally leads to downregulation of E6 and E7, whose products interfere with the p53

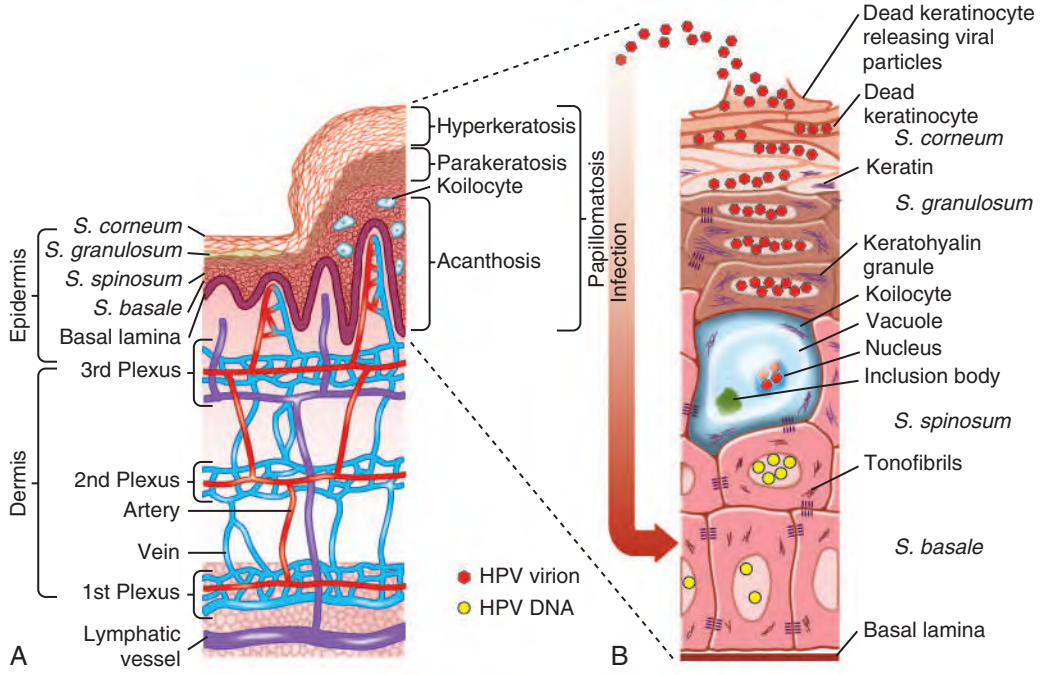


**FIG. 143.1 Exophytic cutaneous wart: human papillomavirus *(HPV)* pathogenesis.** (A) Histologic features. (B) Cytologic features (see text for details). *S.*, stratum.

and retinoblastoma tumor suppressor proteins (see "Virology").[1–4,7–9,12] Nevertheless, the frequency of viral integration varies with the HPV genotype and does not appear to be necessary for oncogenesis. Other events are also important, including hypermethylation of viral and cellular DNA; inhibition of apoptosis and telomerase activation, which both confer immortality; and cooperation with activated cellular oncogenes. The development of chromosomal instability and deletions (6p, 3p, 4p, 6q, 10p, and ultimately 11q) occur as the lesion becomes a high-grade intraepithelial neoplasia.[a]

Host defense responses to HPV infection are not fully understood.[87–93] Nevertheless, several clinical observations indicate that an effective immune system is important in the resolution of HPV infection. Epidermodysplasia verruciformis is a genodermatosis that results from the inactivation of two genes, *EVER1/TMC6* and *EVER2/TMC8*, that code for endoplasmic zinc transporter proteins, components of the nuclear factor kappa B cascade regulating the immune system. Primary immunodeficiencies, such as SCID, common variable immunodeficiency, Wiskott-Aldrich syndrome, and ataxia-telangiectasia, are known for their association with verrucosis, but there are many more.[94] For example, the WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome results from a gain-of-function mutation in the *CXCR4* chemokine receptor gene. Other entities include WILD (warts, immunodeficiency, lymphedema, and [anal] dysplasia) syndrome, XHIGCM1 (X-linked hyper–immunoglobulin M syndrome type 1), Netherton syndrome, and mutations in either the dedicator of cytokinesis 8 *(DOCKS8)* or of the *GATA2* genes.[95] Idiopathic CD4 lymphopenia has been associated with some of the most dramatic cases of profuse verrucosis, the so-called "treeman" patients. Severe frequent HPV disease is also seen in patients with lymphoproliferative disorders and in those with HIV infections.[96–102] The range of HPV-related diseases in HIV infection includes cutaneous warts, anogenital warts, CIN in women, and anal intraepithelial neoplasia and cancer in men who have sex with men (MSM).[96,102–104] HIV infection increases by 4-fold to 40-fold the incidence and prevalence rates of genital warts and CIN.[103] The prevalence of these conditions is greater with low counts of $CD4^+$ T lymphocytes and high HIV-1 RNA levels. Compared with subjects with HIV seronegativity, patients with acquired immunodeficiency syndrome (AIDS) have an increased risk for developing in situ or invasive SCCs of the cervix, vulva-vagina, anus (both genders), and penis.[100,105,106] Contrary to the expectation that led to the inclusion of cervical cancer as an AIDS-defining illness, the progression to AIDS does not appear to augment dramatically the risk of cervical cancer, even if a low $CD4^+$ T-lymphocyte count is a risk factor.[105–109] In contrast, the risk of invasive cancer of the anus in men and in situ and invasive cancers of the vagina or vulva do increase with AIDS progression, as well as with a low $CD4^+$ T-lymphocyte count at the time of AIDS onset.[108] The increased risk of anal cancer is greater than 40-fold.[110] Immunosuppressive therapy, notably in renal allograft recipients, has also been associated with high rates of extensive HPV infection.[98,99,111,112] Another indication of the role of the immune system comes from the observation that the regression of a wart may be promptly followed by the spontaneous regression of others.[113,114] Although the relative immunosuppression of pregnancy appears to be associated with an increased incidence and severity of HPV disease,[43,115] rates of HPV infection are not clearly found to be substantially higher in this population than in nonpregnant women.[116–118]

Evidence also shows that HPV-16 E6 and E7 proteins may inhibit Toll-like receptor 9 (TLR9), a component of innate immunity.[119]

The keratinocytes possess various TLRs, in particular TLR9, that recognize foreign pathogen-associated molecular patterns.[120] This causes the production of numerous cytokines and chemokines, including tumor necrosis factor-α; monocyte chemotactic protein-1; chemokines CCL2, CCL20, and CXCL9; CXCL10 vascular endothelial cell growth factor; interleukins 5 and 8; retinoic acid; transforming growth factor-β; interferons (IFNs) α, β, and γ; and IFN-γ–inducible protein-10.[2–4,8,9,12,121] Overall, this biochemical activity is associated with increased leukocyte trafficking and angiogenesis.

Concomitantly, at the histologic level, an alteration is seen in the number and function of the natural killer and helper T cells and cutaneous Langerhans cells.[122,123] These cells contribute to the local and systemic immune response, which becomes more apparent in regressing warts, as they show a clear lymphomononuclear cell infiltrate.[124]

A humoral and cellular immune response does develop after HPV infection, but its laboratory correlates are not necessarily uniform or constant. The E7 and L1 proteins are the strongest antigens.[125–127] The

[a]References 2, 3, 4, 8, 9, 12.

enhancement after immunization of neutralizing antibody formation against the L1 native protein has been exploited to produce the currently available vaccines (see "Prevention").

HPV also interacts actively with the immune response.[128] For example, HPV E7 reduces the expression of the chemokine CXCL14, which would otherwise trigger the recruitment of natural killer cells, T cells, Langerhans cells, and dendritic cells. HPV E6 and E7 downregulate the IFN response, the expression of TLR9, and alter the cGAS-STING (*c*yclic *G*MP-*A*MP *s*ynthase–*st*imulator of *in*terferon *g*enes) sensing pathway defending against DNA viruses. These viral proteins, along with E5, interfere with antigen processing at the level of intracytoplasmic transport and peptide loading on the cell surface major histocompatibility complex proteins.

Immunogenetic factors are also important. In a large case-control study of the association of classes A, B, and C human leukocyte antigen (HLA); DRB1 and DQB1 alleles; and cervical SCC, several low-magnitude (about twofold or less) associations were observed.[129] For example, class I HLA allele A*0301 increased the risk, and B*1501 decreased it. Similarly, class II HLA allele DQB1*0301 was a risk, and DRB1*1302 was protective. A particular combination of alleles, B*4402-DRB1*1101-DQB1*1302, increased the risk 10-fold. The same immunogenetic risk factors were noted with cervical adenocarcinoma and vulvar SCC. These results confirm the important role that helper and cytotoxic cell responses play in the development of HPV-associated genital cancer.[130]

## CLINICAL MANIFESTATIONS

### Cutaneous Warts

*Cutaneous warts* include deep plantar warts, common warts, and plane or flat warts.[131,132]

*Deep plantar warts (verrucae plantaris)*, also called *myrmecia* (from the Greek, meaning "ant hill"), affect mostly adolescents and young adults. The lesions characteristically look like deep-seated, raised bundles of soft keratotic fibers 2 mm to 1 cm in diameter; shaving reveals punctate, bleeding blood vessels. When grouped in clusters they are called mosaic warts. These lesions are often painful and may also be located on the palms of the hands.

*Common warts (verrucae vulgaris)* appear as well-demarcated, exophytic, hyperkeratotic papules with a rough surface. They may occur on the dorsum of the hand, between the fingers, around the nails (periungual warts), on the palms or soles, or, rarely, on mucous membranes. Warts may coalesce and reach a diameter of 1 cm. Morphologic variants of common warts include mosaic warts, which appear as cobblestone-like patches of aggregated warts several square centimeters in diameter and barely rising above an indurated base. Filiform warts on the head and vegetating, hyperproliferative warts on the hands of butchers, fish handlers, and meat packers also occur.[29]

*Plane warts (verrucae planae)* are commonly found on children and appear as multiple, slightly elevated papules with an irregular contour and distribution and a smooth surface. They occur on the face, neck, and hands. When more protuberant, these lesions are called *intermediate warts*.

Cutaneous warts are usually asymptomatic, although they may bleed and can be painful when located over weight-bearing surfaces or points of friction. Rarely, cutaneous warts may degenerate into verrucous carcinomas.[133] The natural history of cutaneous warts is poorly characterized. Spontaneous resolution appears to occur in 50% and 90% of children within 1 and 5 years, respectively.[26] In a given patient two-thirds of the warts that resolve spontaneously do so within 2 months.[134]

### Epidermodysplasia Verruciformis

*Epidermodysplasia verruciformis* is an autosomal-recessive genodermatosis linked to gene loci on chromosome 17.[2,30] The lesions are associated with a large array of HPV types, some linked to malignant transformation (see Table 143.1), most of which are specific for epidermodysplasia verruciformis.[2,30] These warts have several morphologic variants. They may resemble flat warts but more commonly resemble lesions of pityriasis versicolor, which cover the torso and upper extremities. Over extensor surfaces, these warts may become hypertrophic and coalescent. In most patients, warts appear in the first decade of life. Beginning in young adulthood, in about one-third of patients, the lesions undergo malignant transformation into invasive SCCs, particularly in sun-exposed areas. Although these patients may have depressed cellular immunity,[2,30] they appear to have normal resistance to other pathogens. Epidermodysplasia verruciformis does not appear to be contagious to healthy contacts. Of interest, lesions that resemble epidermodysplasia verruciformis have been observed in HIV-infected patients and solid-organ allograft recipients.[30]

**FIG. 143.2 Vulvar condylomata acuminata.** *(From Gagné H. Colposcopy of the vagina and vulva.* Obstet Gynecol Clin North Am. *2008;35:659-669.)*

### Anogenital Warts

*Anogenital warts* are flesh colored to gray colored, hyperkeratotic, exophytic papules, either sessile on the skin or, more frequently, attached by a short, broad peduncle (Fig. 143.2). Lesions range from smooth, pearly papules to more jagged, acuminate growths. They vary in size from less than a millimeter in diameter to several square centimeters when they merge into plaques. In uncircumcised men the preputial cavity is involved in 85% to 90% of cases.[43,135,136] In the United States, where about 85% of the male population is circumcised, the penile shaft is the most common site of lesions. The urethral meatus is also involved in 1% to 25% of patients.[137] Urethral warts are clearly visible with eversion of the meatus or with the use of a pediatric nasal speculum. They are mostly confined to the fossa navicularis or, less frequently, to the distal 3 cm of the urethra. Involvement of the bladder or proximal urethra is exceptional.[137] Involvement of the perianal area varies according to sexual practice, from very high among MSM (about 10%, and double with HIV seropositivity) to low among heterosexual men.[138,139] Lesions are only occasionally observed on the scrotum, perineum, groin, or pubic area.

In women most lesions are distributed over the posterior introitus and, to a lesser degree, over the labia majora and minora and the clitoris (see Fig. 143.2). In order of decreasing frequency, the perineum, vagina, anus, cervix, and urethra each represent less than one-quarter of the sites of involvement.[43]

The use of the colposcope and prior soaking of examined tissues with 3% to 5% acetic acid has expanded the clinical spectrum of anogenital warts, particularly those caused by HPV types 16 and 18, which can be small acetowhite papules.[140] This technique was initially used to show the existence of flat condylomas on the uterine cervix. Typically, these lesions are shiny white patches with geographic borders

**FIG. 143.3 Pigmented penile warts mimicking bowenoid papulosis.** *(From Habit TP, ed.* Clinical Dermatology. *4th ed. London: Mosby; 2004.)*

and an irregular surface that contains characteristic capillary loops.[141] The presence of external genital warts may indicate the existence of cervical HPV squamous epithelial lesions, including CIN.[142,143] Morphologic differentiation among the grades of cervical squamous epithelial lesions is not sufficiently reliable, and biopsy is strongly recommended for diagnosis.[144,145]

In the vagina, in addition to flat condylomas, small white nodosities centered on a capillary loop, called *spiked condylomas*, have been described.[146] The vulvar introitus may display prominent, sometimes painful papillae whose relation to HPV infection is unlikely but controversial.[147,148] HPV infection of the vulva may also appear as white patches revealed or accentuated with the application of acetic acid, but acetowhitening lacks specificity.[149]

In men acetic acid soaking or examination with a colposcope has shown HPV-infected papules and macules to be up to two times more common than exophytic condylomas, particularly on the prepuce and scrotum.[140,150] With a range in size from minuscule to 1 cm in diameter, round sessile papules with brown to slate-blue pigmentation are encountered on both male (Fig. 143.3) and female external genitalia. These lesions and similarly colored macules are important to recognize because they may represent either HPV-6– or HPV-11–infected benign condylomas,[151,152] seborrheic keratoses,[153] or intraepithelial neoplasias associated with HPV type 16 or 18 infection.[152–154]

About three-quarters of patients with anogenital warts are asymptomatic. Otherwise, itching and burning, pain, and tenderness are encountered frequently. In addition, the disease can have serious psychological effects.[155] The natural history of genital warts, particularly of subclinical HPV disease, is poorly understood, but spontaneous remission may occur, as shown by the results of randomized, placebo-controlled therapeutic trials that indicate up to 10% to 20% spontaneous remission rates in untreated lesions over a 3- to 4-month period.[156–159]

Exophytic genital warts may rarely transform into invasive SCCs, including verrucous carcinoma. They may also reach considerable size, particularly during pregnancy or immunosuppression.[160] When large condylomas reveal histologic features of local destructive invasion without metastases, they may be called *Buschke-Löwenstein tumors*, a term that regroups verrucous carcinomas and giant condylomas.[161,162] A related lesion, condylomatous (warty) carcinoma, may metastasize.[162] Genital HPV infections may also belong to the spectrum of penile, anal, vulvar, vaginal, and cervical intraepithelial neoplasias (PIN, AIN, VIN, VAIN, and CIN, respectively).[163,164] For historical reasons, some variants of intraepithelial neoplasias are further recognized. Histologically, pigmented papules of the external genitalia may show condylomatous cytoarchitecture with evidence of intraepithelial neoplasia.[152] This clinicopathologic entity is called *bowenoid papulosis* (see Fig. 143.3).[165] *Bowenoid papulosis* can evolve to *Bowen disease*, which manifests as a flat red-to-brown plaque with well-demarcated borders and a scaly irregular surface.[166] On the glans penis the lesion is known as *erythroplasia of Queyrat*. Histologically, carcinoma in situ (CIS) is present. HPV-16 and HPV-18 have been recovered from both *bowenoid papulosis* and *Bowen disease*.[167] The natural history of intraepithelial neoplasias is best understood in cervical lesions.[168] Clearly, the outcome (regression, no change, or progression) is highly variable and depends on the histologic grade of the tumor, the HPV type, and the method of diagnosis (conization, punch biopsy, or scraping). CIN grade 1 lesions have an approximate probability of 60% to regress, 30% to remain unchanged, 10% to progress to CIN 3, and 1% to progress to invasive cancer.[168] For CIN 2 the figures are 40%, 40%, 20%, and 5%, respectively. The risk of progression to cancer is the highest with CIN 3 at 12%; only a third of these lesions disappear spontaneously.

Perianal warts are common among homosexual men, and up to two-thirds of patients with external anal warts also have internal lesions.[169] In consequence, the presence of perianal warts or anal symptoms in association with a history of anal sexual play or intercourse should prompt a digital rectal examination and an anoscopic evaluation. After the malignant transformation of anal condylomas was described,[170] the association between anorectal dysplasia or cancer and HPV infection was recognized in MSM.[171,172] Passive anal intercourse carries a risk of anal cancer in MSM, and heterosexual men and women with a history of anogenital warts have a 30-fold increased risk of disease compared with control populations.[173] The anus and the cervix have a different biology respective to HPV. HPV infections in women with and without HIV are 79% and 43% prevalent in the anus, respectively, but only 53% and 25%, respectively, in the cervix.[174] In the general population a history of anal warts increases by about 10 times the risk of anal cancer.[175] During pregnancy HPV shedding may increase, and condylomas may become so large as to impair normal delivery mechanically.[118,160,176] Anogenital warts in children should always raise the possibility of sexual abuse, but in very young children nongenital or possibly perinatal transmission may be the predominant mode of acquisition.[177–179]

### Recurrent Respiratory Papillomatosis

*Recurrent respiratory papillomatosis* has been described by several authors.[37,180,181] Patients present with hoarseness or, in infants, with an altered cry. Sometimes these symptoms are accompanied by respiratory distress or stridor. The disease may spread to the trachea and lungs and lead to obstruction, infection, and respiratory failure. In young children rapid growth of lesions often threatens the upper respiratory tract and frequently necessitates surgical excision to avoid asphyxiation. In adults the course of the disease is usually less aggressive. Lesions may, however, undergo malignant transformation, particularly in patients who have received radiation therapy or in cases with lung involvement.

### Other Human Papillomavirus Infections

Oral squamous cell papillomas (or squamous papillomas) are the most common HPV-related oral lesions. A closely related entity, with slightly different histologic features, is oral condyloma acuminatum. Both types of lesions are caused by mucosal HPV (mostly HPV-6, HPV-11, and HPV-16). Oral verrucae vulgaris are rarer and can be differentiated reliably only with histology. They are caused by cutaneous HPVs (HPV-2, HPV-4, HPV-57).[182] Focal epithelial hyperplasia of the oral cavity (Heck disease) is caused predominantly by HPV-3 and HPV-13 and tends to regress spontaneously.[183] Other HPV infections may also occur in the oral cavity.[183] The oropharynx is now the most common site for the development of HPV-associated cancer.[69] Conjunctival HPV-related papillomas and SCCs, and periungual SCCs have been described.[64] HPV DNA has also been identified in other skin lesions, such as epidermoid cysts, seborrheic keratoses (especially vulvar), skin squamous cell and basal carcinomas,[22] and aerodigestive carcinomas. The prevalence of HPV in these different lesions varies, which makes a causative link difficult to establish.

## DIAGNOSIS

The diagnosis of warts is usually made clinically with physical examination. Exophytic warts have a characteristic appearance. Deep plantar warts may be confused with calluses, but paring usually reveals typical punctate, thrombosed capillaries. Nevi, seborrheic keratoses,

acrochordons, acanthomas, molluscum contagiosum, lichen planus, syringomas, and dermofibromas may be confused with cutaneous warts. Lesions of epidermodysplasia verruciformis may be similar to those of flat warts or pityriasis versicolor, but the patient's history should clarify the diagnosis.

Condyloma acuminatum of the external anogenital tract should rarely be confused with other STDs, such as condyloma latum of syphilis, nodular scabies, genital herpes, lymphogranuloma venereum, chancroid, or granuloma inguinale. Nevertheless, molluscum contagiosum, particularly in its more atypical presentations, may be difficult to distinguish from anogenital warts. In contrast to those of condyloma acuminatum, the lesions of molluscum contagiosum tend to predominate over the pubis and are rarely pedunculated, but rather appear as smooth, sessile domes, the color of the skin or lighter, often with a depressed center from which cheesy material can be expressed. In men a normal anatomic variant of the corona, hirsutoid papillomatosis (pearly coronal papules, papillae corona glandis), can be difficult to differentiate from small warts. A similar anatomic presentation exists in the vulvar introitus, where lesions may appear identical to those of HPV-related vulvar papillomatosis. On the keratinized vulva, hidradenoma papilliferum may be confused with a large wart. On the scrotum, epidermoid cysts and angiokeratomas should be easy to identify. Small and flat HPV lesions may sometimes be difficult to distinguish from lichen planus, lichen sclerosus et atrophicus, lichen nitidus, or syringomas, even with the help of the colposcope and acetic acid application. Finally, pigmented HPV lesions may be confused with nevi or seborrheic keratoses (see Fig. 143.3).

Although initially designed for the evaluation of the female internal genital tract, the colposcope, with prior application for 3 to 5 minutes of a 3% to 5% acetic acid solution, has become an important diagnostic tool for other HPV infections as well.[184] In studies of male partners of women with either cervical condylomas or dysplasias, biopsy-proven genital condylomas were detected in 65% to 88% of the patients, respectively. More significant, 43% to 73% of the lesions were seen only with a colposcope, whereas acetowhitening alone was used for the diagnosis in 22% of patients.[140,185,186] The same technique applied to the vulva revealed subclinical papillomavirus infection in 96% of women with vulvar warts and 80% of women who were partners of men with penile warts.[187] In the oral cavity 83% of HPV lesions are seen only with the colposcope.[188] The clinical significance of lesions that are detectable with acetowhitening only is unknown, and acetowhitening lacks specificity for the diagnosis of HPV infection, particularly for external anogenital warts.[153,189,190]

Lesions of the external genitalia that are pigmented (see Fig. 143.3), appear as plaques, bleed, or are large should have biopsies to establish the diagnosis and rule out malignancy.[152] Biopsy is also indicated to confirm the diagnosis of epidermodysplasia verruciformis and to determine the cause of lesions of the oral cavity and upper airways.

Anoscopic examination should be considered in patients with perianal warts, anal symptoms, or a history of receptive anal intercourse. Most intraanal lesions are below the pectinate line, and sigmoidoscopy is not routinely indicated.[191,192] The oral cavity should preferably be examined in all patients with anogenital warts because of the possibility of concomitant oral warts.[188]

Evaluation of the vagina and cervix, when appropriate, should include colposcopy and acetic acid application and should seek to rule out invasive cancer.[184] Internationally applicable colposcopic terminology should improve diagnostic accuracy and reliability.[141] Women with a history of anogenital HPV disease or whose sexual partners have had anogenital HPV disease should have a cytologic examination of a cervical smear (Pap smear), at least as part of regular screening (see "Prevention"). Koilocytes on a cytologic smear are the hallmark of HPV infection.[193] More important, diagnoses of dysplasia and cancer can also be made from the smear.[194] Depending on the patient's age and the location and nature of the HPV infection, the sensitivity of the Pap smear in detection of HPV infection ranges from 30% to 90%.[195]

The use of the colposcope during the anoscopic examination (high-resolution anoscopy [HRA]) combined with anal cytology has been applied with success to the diagnosis of intraanal HPV lesions.[196] It can be a screening tool for anal intraepithelial neoplasias in homosexual or bisexual males or in the female with HIV.[171,174,196–198] So far, only New York State recommends anal cytology and HRA for the management of women with HIV with high-grade intraepithelial neoplasia and of any patient with HIV with abnormal anal physical findings (www.hivguidelines.org). However, cytology turned out to have poor specificity in the given patient population, thus increasing the reliance on histology, and possibly HPV DNA testing, and high-grade AIN recurrence is high.[198,199] Nevertheless, any of these approaches have yet to be validated with long-term studies of outcomes (anal cancer), reliability, safety, and costs. In consequence, they have not been endorsed by broad public health policies.[200,201] Automated, liquid-based collection for cervical cytology has largely supplanted the conventional Pap smear in the United States.[194,202] Its only, but significant, advantage is that it allows, if need be, HPV DNA testing on the same sample (so-called reflex testing), which is now part of the screening strategy for cervical cancer.[202,203]

Cervical cytology has benefited from the development of the Bethesda system, last revised in 2002.[204] This interpretation scheme addresses the adequacy of the specimen, classifies its pathologic features, and provides guidelines for management and follow-up, updated in 2012 (http://www.asccp.org/asccp-guidelines).[204,205] HPV-related squamous cell abnormalities are regrouped in four categories: (1) atypical squamous cells "a" of undetermined significance (ASC-US) or "b," for which a high-grade squamous intraepithelial lesion (ASC-H) cannot be excluded; (2) low-grade squamous intraepithelial lesion (LSIL), a diagnosis that regroups the previous cytologic and histologic diagnoses of koilocytic or condylomatous atypia, mild dysplasia, and CIN 1; (3) high-grade squamous intraepithelial lesion (HSIL), previously including moderate and severe dysplasia, CIN 2 and CIN 3, and CIS; and (4) SCC.

The general histopathologic features of HPV infection are usually characteristic (see "Pathogenesis"). Therefore biopsy can be used to confirm most diagnoses. In addition, histologic examination can identify the presence of intraepithelial neoplasia or invasive cancer. Although histology is the gold standard, like cytology it suffers from lack of accuracy and reliability where disease grades are concerned.[194,206]

To enhance the sensitivity and specificity of cytohistopathology, two types of techniques are now available to the clinical laboratory. They rely on the demonstration in the cytologic or biopsy specimens of HPV nucleic acids or cellular antigens indicative of oncogenic risk.[207–209]

Among the approximately 200 commercially available tests, seven HPV nucleic acid detection tests are approved by the US Food and Drug Administration (FDA).[210] They have at least one of three purposes: the primary screening of cervical cancer, the reflex testing of specimens diagnosed as ASC-US for further identification of the women requiring colposcopy, and the management of women 30 years and older by doing contesting with cytology.

The tests are available on automated platforms. The Hybrid Capture II High Risk DNA (Quiagen, Germantown, MD) detects with RNA probes the DNA of 12 oncogenic HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, and 68). It does not differentiate the type(s) identified and does not check for the presence of human DNA. Cervista HPV HR (Hologic, Marlborough, MA) targets the HPV E6/E7 DNA of the same types as of the Hybrid Capture II as well as HPV-66. It relies on signal amplification, and assesses the presence of human cells in the specimen. The result does not differentiate the HPV type(s) present. A related test, Cervista HPV16/18 (Hologic), identifies whether types 16 or 18 are present. The COBAS 4800 HPV (Roche, Indianapolis, IN) is a polymerase chain reaction (PCR) assay targeting the L1 region of the same 13 types as the Cervista HPV HR assay. It incorporates a human β-globin control. Results are given as to the presence of HPV-16, HPV-18, or others. This assay is the only one approved for primary screening. The APTIMA HPV (Hologic) is an assay that detects the E6/E7 transcripts of the same HPV types as the COBAS assay, plus those of HPV-56, by transcription-mediated amplification. Detection of the amplification products is done by a hybridization protection assay. It includes a β-globin control. The result does not differentiate among the genotypes. A similar assay, the APTIMA HPV 16, 18/45 genotype assay does identify if HPV-16, HPV-18, and/or HPV-45 are present. A seventh assay, ONCLARITY HPV (Becton Dickenson, Franklin Lakes, NJ) detects the *E6/E7* DNA by real-time PCR. It has a β-globin control.

It reports the individual detection of HPV genotypes 16, 18, 31, 45, 51, and 52, and the group detection of 33/58, 35/39/68, or 56/59/66. These different assays, although not identical in their performance characteristics, appear equivalent in clinical effectiveness.[211–214]

Under the stimulus of HPV E6 or E7, lesions that progress to cancer also produce in greater quantity various proteins associated with the host cell-cycle regulation. These proteins can be detected by immunocytohistochemistry and provide the pathologist with additional prognostic tools.[215–218] Among these biomarkers, $p16^{INK4A}$ has received the most attention, and it has been integrated in the histologic diagnostic approach.[209,219] It is possible to identify on tissue sections the presence of the papillomavirus common antigen by immunocytochemistry.[19]

Virus cultivation techniques are not available for the clinical diagnosis of HPV infections. HPV infection may elicit a serologic response. In patients with cutaneous warts, condyloma acuminatum, or recurrent respiratory papillomatosis, antibodies directed against the viral capsid have been detected.[126] Recombinant VLPs based on the L1 or L1 and L2 proteins offer the same antigenic properties as viral capsids.[126] They have been used extensively to show, with enzyme-linked immunosorbent assay, that about one-half to almost 90% of patients with HPV infection have capsid antibodies.[126] Anti-HPV antibodies tend to disappear with disease resolution but can persist for several years in asymptomatic patients.[126] A fraction of the antibodies produced in response to HPV infection are neutralizing but in amounts offering at best limited protection sufficient to offer significant protection.[220,220a] No commercial assays are available for the serologic diagnosis of HPV infections because of insufficient sensitivity and clinical specificity. Such assays have been used for seroepidemiologic surveys. Moreover, assays that measure binding and neutralizing activity against the viral capsid have been useful in assessment of the immune response after HPV vaccination (see "Prevention").

## THERAPY

Highly effective and safe treatments for HPV diseases are not yet available, and the current therapies are not designed to eradicate HPV infection. Rather, their purpose is to decrease or, if possible, eliminate clinical manifestations. The current therapeutic armamentarium has been largely developed empirically over decades and too often relies on the physical or chemical destruction of lesions. Newer approaches are directed at molecular viral targets and immunomodulation.[13,221–223]

### Cutaneous Warts

The choice of treatments for cutaneous warts is complicated by the existence of weak and confusing evidence.[224–226] Nevertheless, the most common approach, the topical application of preparations that contain salicylic acid, a keratolytic agent, is effective for the treatment of common warts.[224–226] A meta-analysis of five placebo-controlled clinical trials revealed a complete response rate of 71% (117 of 165) in the cases, compared with 47% (78 of 166) in the control groups.[224,225] A widely available over-the-counter preparation for self-treatment is a salicylic acid and lactic acid paint (salicylic acid, lactic acid, collodion, 1:1:4 [SAL]) that is typically applied daily for up to 12 weeks. The cornified layer that typically covers skin warts may need to be removed. This removal is done with a hot water soak, followed by abrasion with a pumice stone, sandpaper, or an emery board. Occlusive bandages seem to increase treatment effectiveness. Mosaic warts tend to be more resistant to treatment than myrmecia.

Cryotherapy is a popular treatment, but it requires a health practitioner. It is typically accomplished with cotton wool buds dipped in liquid nitrogen and applied to the lesion or with spraying of liquid nitrogen.[227,228] Randomized, placebo-controlled studies have been inconclusive on the efficacy of cryotherapy, but when compared with salicylic acid preparations, cryotherapy appears to be equivalent.[224,225] Variations in technique may account for these confusing results. However, aggressive cryotherapy, a 10-second sustained freeze, is more effective than briefer traditional cryotherapy, despite a higher incidence of pain and blisters.[224,225,229] More than one treatment is often needed. A 2-week interval offers the best balance between the occurrence of side effects and brevity of treatment.[230] Treatment beyond 3 months, or about four cryotherapies, presents little advantage.[231]

A randomized study with blind evaluation compared cryotherapy with duct tape application, an occlusive treatment that has long been in the medical lore, for the treatment of common warts.[232] Complete clearance of the warts occurred in 85% (22 of 26) of the patients who received occlusive therapy but in only 60% (15 of 25) of the patients treated with cryotherapy. However, this trial had limitations with blinding and follow-up. Two subsequent placebo-controlled trials of duct tape application produced negative results.[233,234]

Other treatment methods that are less often used include glutaraldehyde, formaldehyde, podofilox, and cantharidin. Their use is empirical. Intralesional bleomycin has been better studied, but when it is compared with placebo, the results are inconclusive.[224] It is usually reserved for the treatment of periungual warts. Other treatments reportedly superior to placebo include silver nitrate sticks and topical zinc sulfate preparations.[226,235,236]

Allergic sensitization with dinitrochlorobenzene (DNCB), followed by direct application of DNCB on the lesions, has been found to be twice as effective as placebo.[224,237,238] However, the use of DNCB is risky, and other sensitizing agents, such as 2,3-diphenylcyclopropenone, squaric acid dibutyl ester, and 10% masoprocol cream (Actinex, Schwarz Pharma, Mequon, WI), appear to be safer and as effective.[237,238]

Imiquimod is an immunomodulator that is approved by the FDA for the topical treatment of genital warts (see "Anogenital Warts"). When used off label in an open study, imiquimod 5% cream applied once a day, 5 days per week, for up to 16 weeks on varied common warts resulted in a complete response.[238]

Cimetidine, an H2 blocker that has immunomodulatory properties, has been widely publicized as an effective treatment for cutaneous warts on the basis of uncontrolled studies. Yet several placebo-controlled, double-blind studies have failed to confirm that claim.[237,238]

Electrosurgery and laser surgery are used, but they can be expensive and have not been rigorously evaluated.[224,237,239,240] Electrosurgery is relatively contraindicated for the treatment of plantar warts because of the risk of permanent and painful scarring. Laser surgery is also not scar-free, but it may be useful for the treatment of periungual and subungual warts.

Photodynamic therapy, which relies on laser light to activate locally the cytotoxicity of a compound administered systemically or applied topically, is superior to placebo for the treatment of cutaneous warts.[224] However, the technique is costly and not widely available.

Suggestion, hypnosis, homeopathy, and distant healing are among alternative approaches that have been proposed for the treatment of cutaneous warts.[241–245] More rigorous evaluations of these interventions showed little, if any, promise.

Particular treatment methods have been proposed for some specific types of warts. For example, flat warts rarely need treatment, but when they do, cryotherapy or electrosurgery (electrodesiccation) is used. Cryotherapy may also be used for the treatment of eyelid and periungual warts, and electrodesiccation is useful to remove flat or filiform warts.

### Anogenital Warts

The treatment methods for condyloma acuminatum are numerous yet unsatisfactory, but guidelines that attempt to optimize the therapeutic approach have been published.[201,246–250] Because no or scant evidence shows that treatment directly affects eradication of HPV, transmission of infection, or prevents the uncommon development of neoplasms, the rationale for treatment is restricted.[85,251,252] It includes cosmesis, relief of local symptoms, alleviation of the adverse psychological impact caused by the presence of anogenital warts,[155,253–257] and restoration of normal physiologic function (e.g., debulking of lesions that obstruct the birth canal). Before treatment is initiated, the goals of therapy, alternatives, costs, and potential side effects should be discussed with patients. Also, within 3 to 4 months, approximately 10% to 20% of patients have spontaneous resolution of the disease.[156–159,246] Independent of treatment, patient counseling is part of management.[136,258]

None of the available treatment methods is dramatically superior to the others, but each may have its particular advantages. Because convenience is one of the greatest advantages, the availability over the past few years of patient-applied therapies—podofilox, imiquimod, and Veregen (polyphenon E)—has been of considerable interest.

Podofilox (podophyllotoxin) is a derivative of podophyllin, which was long the mainstay of genital wart treatment by practitioners. Podophyllin, a resin extract from the rhizome of *Podophyllum peltatum* (podophyllum resin [US Pharmacopeia]) or *Podophyllum emodi*, has been the principal mode of therapy for many years.[259,260] The active molecules are lignans, particularly podophyllotoxin. Although podophyllin is a mitotic poison, its mode of action in warts is unknown. The compound is usually applied as a 10% to 25% solution in benzoin, directly on the wart, once weekly. Washing of lesions within 12 hours is recommended to minimize local reactions. Lack of regression after four applications suggests the need for alternative therapy. Podophyllin has never been compared with a placebo. Its effectiveness has been evaluated in a series of randomized controlled trials against other treatment methods; complete clearance rates ranged from 20% to 40%, taking into account frequent recurrences.[135,259] Side effects are both local and systemic.[259,260] Chemical burns are seen in one-third to one-half of the patients. Transient pseudoneoplastic histopathologic changes have also been reported. Neurologic, hematologic, and febrile complications, sometimes leading to death, and allergic sensitization have been associated with administration of topical podophyllin. Therefore areas larger than 10 $cm^2$ should not be treated. The drug is contraindicated in pregnancy.

Podophyllotoxin is available in the United States under the generic name podofilox. It offers distinct advantages over podophyllin and is preferentially recommended.[261] It is chemically uniform and of standardized potency. Podofilox is also more efficacious and less toxic than podophyllin.[135,260,262,263] Finally, it does not need to be washed off. Randomized controlled studies have shown that 0.5% podofilox solution applied twice daily for 3 consecutive days every week for up to 4 weeks results in rates of complete response from 45% to 58%.[246,250,263–266] A gel formulation is easier to apply without spillover. Side effects are mostly mild and similar in nature to those of podophyllin. As with podophyllin, relapses are common and occur in 33% to 91% of patients.[246,250,263–266] Application of podofilox to prevent recurrences is effective and well tolerated, but the long-term outcome after cessation of treatment is unknown.[267] In addition to podofilox 0.5% (Condylox; Actavis, Parsippany, NJ) solution, a 0.5% gel is also available. It yielded a 45% (81 of 181) complete clearance rate after 8 weeks in a large randomized controlled trial, as opposed to 4% (5 of 93) for the vehicle only.[268]

Imiquimod is an imidazoquinoline amine that induces the production of IFN-α and other cytokines. It appears to exert its unique antitumor and antiviral action by binding to the TLR7, and possibly TLR8, of dendritic cells.[269] It is available as a 5% cream (Aldara; 3M Pharmaceuticals, St. Paul, MN) and, since 2011, as a 3.75% cream (Zyclara; Graceway Pharmaceuticals, Bristol, TN) for the self-treatment of condyloma acuminatum.[270,271] Imiquimod 5% cream was compared with vehicle alone in a randomized double-blind trial and was given three times per week, on alternate days, for up to 8 weeks.[272] At the end of the treatment period, 108 patients were evaluable, and the complete response rate was 37% in the imiquimod group compared with 0% in the control group ($P < .001$). Nineteen percent of the patients had a recurrence during the 10 weeks of follow-up. In a similar study the treatment duration was extended up to 16 weeks, and imiquimod 5% cream was compared with a 1% cream and with vehicle.[273] At the end of treatment the complete response rates were 50%, 21%, and 14% in the three respective groups. Imiquimod 5% cream was significantly superior to either of the two other preparations ($P < .001$). In the 5% imiquimod group, 72% of women had a complete response, compared with 33% of the men. During the 12 weeks of follow-up, recurrences were noted in 13%, 0%, and 10% of the subjects in the three groups, respectively. The adverse reactions were local and included itching and burning sensations, erythema, erosions, and swelling; they were well tolerated. The daily administration of imiquimod 5% cream offers some enhancement of efficacy, mostly in men, but a substantially higher incidence of side effects.[274] Therefore Aldara is approved for three-times-weekly use only. Additional clinical trials have complemented and supported the results of these pivotal studies.[249,275,276]

The 3.75% cream formulation (Zyclara) is designed to be administered daily for up to 8 weeks.[271] This formulation was compared with a 2.5% formulation and a placebo in two randomized clinical trials that included 534 women. At 12-week follow-up, the complete response rates were 43.1% for the 3.75% cream, 35.1% for the 2.5% cream, and 16.1% for the placebo ($P < .003$ when comparing each of two active groups with the placebo). Rates of drug discontinuation for safety reasons were 2.3%, 1.4%, and 0.9%, respectively, with decreasing drug concentration.

Imiquimod also appears to be useful for the treatment of other possibly HPV-related conditions, such as actinic keratoses, basal cell carcinomas, and SCCs in situ.[222,277]

Veregen (Doak, Fairfield, NJ) is a botanical derived from green tea. It contains sinecatechins (polyphenon E), compounds with cytotoxic and apoptotic properties.[278,279] It is available as a 15% ointment that is self-applied three times per day on the lesions until complete disappearance, but for no more than 16 weeks. Three randomized controlled clinical studies have been conducted in men and women with genital warts, with a total of 477 subjects in the 15% ointment arm and 290 controls.[280] In the aggregate the complete clearance rate was 56% with the active compound and 37% in the placebo arm. Efficacy was better in women than men. The side effects were local and included erythema (18%), pruritus (14%), pain (14%), and ulceration (12%).[281] The drug is contraindicated in pregnancy. The red stain of the substance and its frequency of administration are potential drawbacks.

Various provider-applied therapies are available. They can be divided into nonsurgical and surgical treatments, which are as follows.

Podophyllin resin (see previous discussion) is still used widely where cost is an issue, although podofilox 0.5% solution or gel is more effective and safer to use.[260]

Trichloracetic acid (TCA) and, to a lesser extent, bichloracetic acid have been favored by gynecologists for the treatment of genital warts.[282] They can both be used during pregnancy. TCA in a 10% to 90% solution is used topically at weekly intervals. The application is painful and can cause ulcers. The unreacted acid should be removed with talcum powder or bicarbonate of soda. In one comparative trial TCA therapy appeared to be equivalent to cryotherapy, with complete response and relapse rates of 81% and 36%, respectively.[283] Another study was also unable to detect any differences, with complete response rates of 64% for cryotherapy and 70% for TCA.[284] TCA at 50% does not add to the effects of podophyllin alone and is ineffective in the treatment of vaginal and cervical warts.[285,286]

Cryotherapy is administered with a liquid nitrogen spray or cryoprobe. Lesions are frozen every 1 or 2 weeks. Cryotherapy is regarded as an effective treatment, with cure rates in the 50% to 100% range, and it is safe even during pregnancy.[283,287] One comparative study suggested that cryotherapy is more effective than podophyllin but probably less effective than electrosurgery.[288–290] Side effects are tolerable and include burning, which resolves within a few hours, and ulceration, which heals in 7 to 10 days with little or no scarring.

Other surgical techniques are available for the treatment of anogenital warts.[201,249,250] Conventional surgery with scissors offers the advantage of immediate eradication of visible lesions. This technique has been reserved mainly for the treatment of perianal warts, but it can be advantageously applied to other genital warts if they are limited in number. Up to one-third of patients have recurrences, and scarring, typically limited to some skin discoloration, is the most common complication.[291–294] Electrosurgical techniques have often been applied for the treatment of external genital warts, with results probably superior to those of cryotherapy, but scarring may occur.[289,290] Complete response rates of 80% to 90% have been reported with $CO_2$ laser therapy.[295–297] In a comparative assessment, however, laser therapy was not deemed to be superior to conventional surgery,[292] and subsequent better-designed studies indicated a long-term complete response rate of 19% to 39%.[298,299] Laser therapy is expensive, may require general anesthesia, and is frequently accompanied by pain and scarring.

The availability of lidocaine-prilocaine (EMLA) cream, which should be applied about 1 hour before the procedure, has facilitated local anesthesia before cryotherapy and laser surgery.[300–303]

Two treatments that are now rarely used but still deserve mention are 5-fluorouracil (5-FU) and intralesional IFN. 5-FU, used topically as a 5% cream applied daily, has been reported to have cure rates of 30% to 95%; the best results have been obtained with intraurethral

warts.[135,304,305] In a comparative trial in men, 5-FU appeared to be equivalent in efficacy to podophyllin.[306] In addition, prophylactic activity of 5-FU has been reported for vulvar warts.[307] This drug is not widely used because it often produces substantial pain, ulceration, and, if applied in the urethra, dysuria.[135] Like other antimetabolites, 5-FU is contraindicated during pregnancy.

IFNs have antiviral, immunomodulatory, and antiproliferative properties.[308,309] Encouraging in vitro and preliminary clinical studies were confirmed by four randomized, double-blind trials that showed the efficacy of intralesionally administered IFN-α and IFN-β compared with placebo.[113,310–312] Parenterally administered IFNs have also been evaluated for treatment of condyloma acuminatum but have generally been ineffective.[156–158,313] IFN, in the doses used, has been generally well tolerated. Side effects (influenza-like symptoms, neutropenia, and thrombocytopenia) are usually mild and are seen more frequently with higher doses. Imiquimod, an IFN-α inducer, is a more practical and cheaper substitute for IFN. No published experience is available with pegylated IFNs.

Cidofovir is an acyclic nucleotide that is a potent inhibitor of the DNA polymerase of cytomegalovirus (CMV) and other herpes viruses and is licensed for the intravenous treatment of CMV retinitis. Although HPVs do not possess a DNA polymerase, this compound triggers the apoptosis of HPV-infected cells.[222,314] In a randomized, vehicle-controlled trial of a compounded 1% gel applied daily to genital warts for 5 consecutive days every other week, at 12 weeks the treated group had 47% (9 of 19) complete clearance compared with 0 (0 of 11) in the vehicle group ($P = .006$).[315] Pain, pruritus, rash, erosions, and ulcerations were frequently noted but equally in both groups. The cost, the risk of nephrotoxicity, neutropenia, and carcinogenesis associated with cidofovir, and the absence of long-term data are reservations about this non–FDA-approved treatment.[222]

Although guidelines are helpful, firm recommendations on the proper treatment strategy for condyloma acuminatum are not always possible. The divergent results of several cost-benefit analyses reinforce this point.[260,316–318] Costs may vary widely for a given therapy, recurrences are common, yet long-term outcomes are not well studied, and the significance of the antecedent genital wart history and treatment is poorly known. Furthermore, the importance of factors such as gender, wart location, size, and number is largely unknown with respect to each treatment. Nevertheless, the duration of lesions (>1 year), their number (>10), and their location on dry rather than moist skin are adverse predictors of treatment response.[249,319,320] Treatment response may improve with the discontinuation of oral contraceptive use, pubic hair shaving, and tobacco smoking.[321,322]

In practice, availability, convenience, adverse reactions, location of lesions, and characteristics of the patient are determinant in the treatment choice. Patient-applied therapies should receive preference. Warts of the urinary meatus can be treated with careful application of podophyllin, podofilox,[135] or cryotherapy.[323] 5-FU cream may also be used.[135,324] Laser surgery and instillations of IFN-α can also be used with intraurethral warts.[150,325] Perianal and anal warts may be treated with scalpel removal,[291–293] cryotherapy,[326] laser surgery,[327] trichloracetic or bichloracetic acid,[201] or even, as adjunctive therapy, with imiquimod-soaked anal tampons.[328]

For vaginal warts, cryotherapy (sprays), TCA, and podophyllin are simple options[329]; laser therapy[330,331] and cryotherapy[332] have the advantage of being relatively safe during pregnancy, and they may be used for treatment of cervical warts also. Although intralesional IFN may be indicated for the treatment of single, very large warts, laser therapy seems to be better suited for large, extensive lesions.

Although HPV can be transmitted to the neonate and may lead to the development of recurrent respiratory papillomatosis, the presence of genital warts is not an indication for cesarean section because laryngeal papillomatosis is rare and when the transmission of the infection occurs remains uncertain.[37,201]

The genital warts of immunocompromised patients, including those with HIV, may still spontaneously regress, but they tend to be relatively refractory to treatment and frequently relapse.[333–335] Thus podophyllin, podofilox, intralesional IFN, and imiquimod alone have been largely ineffective.[336–340] Combination therapy appears more successful, such as electrosurgery plus cold-blade excision[336,341] or plus intralesional IFN for anal warts.[342] Imiquimod may also be used as adjunctive therapy.[343] Nevertheless, single therapy, especially for small (<1 $cm^2$) intraanal lesions, may be effective, as shown with TCA, liquid nitrogen, or the use of an infrared coagulator.[199,344] Lesion healing is generally not a problem after surgery.[345] Further recommendations for the management of HIV patients with HPV infections have been issued jointly by the Centers for Disease Control and Prevention (CDC), the National Institutes of Health, and the Infectious Diseases Society of America (http://aidsinfo.nih.gov/contentfiles/lvguidelines/Adult_OI.pdf), and the New York State Department of Health has issued, in January 2018, its own guidelines for the screening of cervical cancer (www.hivguidelines.org/adult-hiv/preventive-care-screening/cervical-dysplasia-cancer/#tab_0). The effects of active antiretroviral therapy on HPV diseases have been inconsistent but generally modest when present.[346–350]

Because internal genital warts are often associated with genital dysplasias and malignant diseases and because of the special skills and technical resources necessary for proper diagnosis and management, patients with internal lesions should be referred to a qualified specialist.

### Other Warts

The lesions of epidermodysplasia verruciformis should be carefully observed, and any malignant changes should be treated with surgical techniques (cold blade or laser), cryotherapy, or 5-FU ointments.[30] Retinoids in combination with intralesional IFN or calciferol help with the management of the lesions of epidermodysplasia verruciformis.[30,351]

The management of recurrent respiratory papillomatosis is complex.[37,180,352–354] For the primary debulking of lesions, most surgeons use the $CO_2$ laser. Mechanical devices such as a microresector are also used. Photodynamic laser therapy is gaining acceptance. The recurrent nature of the disease requires a careful balance between the risks and benefits of the surgery, which can be achieved only by experienced and skilled operators. Tracheostomy should be avoided because the papillomatosis could then extend to the tracheostomy site and further down the respiratory tree. Radiotherapy is contraindicated because of the known risk of malignant transformation. Different adjuvant therapies are available. Parenteral IFN-α may yield long-term complete responses in a quarter of patients. The interest moved to the intralesional injection of cidofovir.[222,314,355] However, the excellent results of the early case series precluded the completion of a properly designed study.[314] More recently, severe side effects of nephrotoxicity and neutropenia, and a possible oncogenic risk, have tempered the enthusiasm for use of cidofovir in this setting. Indole-3-carbinol (I3C) and its main active metabolite, diindolylmethane, are derivatives of cruciferous vegetables (e.g., broccoli, cabbage, cauliflower) that are widely used by patients with recurrent respiratory papillomatosis. By increasing the 2-hydroxylation of estradiol, these compounds favor the formation of 2-hydroxyestrone, a nonestrogenic, antiproliferative, antiangiogenic, and apoptotic molecule, instead of 16α-hydroxyestrone. A randomized, placebo-controlled clinical trial has shown the ability of I3C to induce regression of biopsy-proven CIN 2 or 3.[356] A similar trial has not been conducted for recurrent respiratory papillomatosis. Oral warts (squamous papillomas, condylomata acuminata, and verruca vulgaris) can be treated with surgical excision, cryotherapy, laser surgery, or podophyllin application.[357] Because of its benign natural history, focal epithelial hyperplasia should not be treated.

## PREVENTION AND VACCINATION

At present, no effective methods of prevention for cutaneous warts are available, other than avoiding contact with infectious lesions. In the case of plantar warts, empirical evidence suggested that the wearing of protective foot equipment (verruca socks) would be effective, but more recent work argues that the family environment is the more likely source of infection.[41,358–361]

Male condoms offer an imperfect protection against female acquisition of HPV infection.[362] A prospective study of 82 college-aged women, virgins at enrollment, showed protection against HPV cervical infection in direct relationship with frequency of condom use during intercourse.[363] Two randomized trials make a more dramatic argument in favor of condoms by showing that male condom use for at least 3 months

promoted regression of CIN and clearance of HPV DNA in the female sexual partners and regression of HPV-associated penile lesions in the patient.[364,365] This occurred only in couples with concordant HPV types.[366] Therefore reinfection is clinically important. Other epidemiologic data show that consistent condom use halves the risk of HPV acquisition in males.[367]

Examination of the partners provides an opportunity to educate, counsel, and screen for HPV disease and other STDs.[201]

The Pap smear is an essential tool for the screening and prevention of cervical cancer. The latest guidelines for cervical cancer screening from the American Cancer Society (in concert with the American Society for Colposcopy and Cervical Pathology [ASCCP] and the American Society for Clinical Pathology), the US Preventive Services Task Force, and the American College of Obstetricians and Gynecologists are summarized in Table 143.2.[368–370] Consensus guidelines for the management of the cytologic and histologic abnormalities have been issued by the ASCCP (http://www.asccp.org/asccp-guidelines).[201,205,370] Using the cervix as a model, screening for anal cancer using anal cytology has been proposed for populations at risk, such as HIV-infected individuals who are MSM, who have a history of anogenital condylomas, or women with abnormal cervical and/or vulvar histology.[371,372] Unfortunately, anal cytology alone has poor performance characteristics to detect high-grade anal intraepithelial neoplasia (HGAIN) and needs to be augmented by other, more costly tests.[373] Furthermore, there is presently no study results on the impact of HGAIN treatment on anal cancer mortality in HIV patients and its cost. In consequence, no national anal cancer screening guidelines has been issued.[171,374] It is nevertheless recommended to evaluate any symptomatic patient and to perform digital annual rectal examinations in HIV-positive patients and HIV-negative MSM[201] (https://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf).

Recurrent respiratory papillomatosis of children may be acquired by the infant during passage through the birth canal, as discussed previously. Cesarean section has probably only a limited role, if any, in the prevention of respiratory papillomatosis.[37]

## Vaccination

The introduction starting in 2005 of a series of HPV vaccines has marked a great advance in the field of genital HPV diseases and in cancer vaccines in general. These vaccines are all based on VLPs. They are obtained by the expression in eukaryotic vectors of the gene coding for HPV L1, the major capsid protein. The viral proteins self-assemble into noninfectious viral capsids sharing the size, shape, and immunologic characteristics of native, infectious virions. HPV VLPs are capable of inducing neutralizing antibodies at titers sufficiently high and superior to those generated after natural infection, to block HPV infection.[375,376] Protection is entirely dependent on these antibodies and does not rely on cellular immunity.[252,376]

Three HPV vaccines have been licensed. Cervarix (GlaxoSmithKline, London, UK) is made in insect cells and is directed against HPV-16 and HPV-18.[377] This bivalent vaccine is FDA-approved for the prevention of CIN, adenocarcinoma in situ (AIS), and cancer of the cervix associated with these two HPV types. Gardasil (Merck, West Point, PA), which came out first, is made in baker's yeast and is directed at HPV-6, HPV-11, HPV-16, and HPV-18.[378] Since 2016 it has been progressively replaced by Gardasil 9, a nonavalent vaccine that targets five additional high-risk HPV types—31, 33, 45, 52, and 58.[379] Gardasil and Gardasil 9 extend the indications of Cervarix to the prevention of VIN2/3, VAIN2/3, and cancers of the vulva and vagina, and in males to the prevention of AIN and anal cancer. These vaccines differ in their adjuvants. Gardasil and Gardasil 9 contain amorphous aluminum hydroxyphosphate sulfate, whereas Cervarix uses AS04, a combination of aluminum hydroxide and 3-*O*-desacetyl-4′-monophosphoryl lipid A. They are stored by refrigeration but are not frozen, and each dose is given intramuscularly (deltoid muscle) in a volume of 0.5 mL. With the withdrawal of Cervarix, only Gardasil 9 is available in the United States.

Clinical trials, each totaling about 15,000 women aged 16 to 26 years, with CIN2+ and AIS as end point and using a per-protocol population analysis, have shown an efficacy of 98.2% (95% confidence interval [CI], 93.3% to 99.8%) for Gardasil, 42 months after the first dose, and 92.9% (CI, 79.95% to 98.3%) for Cervarix, 35 months after the first dose.[380] The cancer indication has been long supported by the strongly established link between CIN 2/3 and cancer. There is now early direct evidence. In Finland, the incidence of HPV-associated cancers has decreased only in the vaccinated population.[380a] Gardasil also has indications for the protection against VIN/VAIN 2/3, with an efficacy of 100% (CI, 82.6 % to 100%), and against external genital warts, with an efficacy of 99% (CI, 96.2% to 99.2%).[380]

To show that the vaccines are likely effective if administered to children aged 9 or 10 to 15 years, who otherwise are difficult to study because of their very low rate of HPV disease, neutralizing antibody levels were used as a surrogate marker of efficacy and were shown to be higher in boys and girls than in women.[377,378] Intention-to-treat population analyses that allowed the inclusion of women who at entry were not necessarily seronegative and/or HPV DNA negative for the vaccine types showed mediocre or absent vaccine efficacy for disease related to a given HPV type, if the subject was either seropositive or HPV DNA positive for that type.[381,382] However, full efficacy was retained for diseases caused by the other vaccine HPV types. Clearly, vaccination should occur before the onset of sexual intercourse to confer its fullest protection.

Gardasil has now received indications for males up to age 26 years in some populations. In studies conducted in HIV-negative MSM, the vaccine intention-to-treat efficacy was 68% (CI, 48.8% to 80.7%) for external genital warts and 54.2% (CI, 18.0% to 75.3%) against AIN 2/3 caused by HPV-6, HPV-11, HPV-16, or HPV-18. In the HIV-infected population the vaccine is safe and did not affect the $CD4^+$ T-lymphocyte count or the HIV viral load in either adults or children aged 7 to 12 years.[383,384] In these individuals the vaccine induces neutralizing antibodies, albeit at titers about 30% to 50% lower than in non–HIV-infected males. Vaccine efficacy in the HIV population was evaluated in 575 patients (472 MSM and 103 women) aged 27 years or older after immunization with three doses.[385] Although the vaccine was safe and immunogenic, the study was stopped at 2.4 years because it failed to demonstrate benefits regarding persistent anal HPV infections or anal HSIL. This failure was likely caused by the high rates of baseline anal HPV positivity, 60%, and anal HSIL, 33%. This reinforces the need to vaccinate populations early.

Vaccine immunogenicity decreases when administered to older women, both with Gardasil (up to 45 years of age) and Cervarix (up to 55 years of age).[386,387] Gardasil clinical efficacy was also reduced in women aged 24 to 45 years and could only be demonstrated by combining external genital warts and CIN as an end point.[386] Canada has approved Gardasil in women up to age 45 years, but the US Advisory Committee on Immunization Practices has not. This may change now that in October 2018 the FDA has approved Gardasil 9 up to 45 years for both sexes.[386a]

Gardasil 9 registration clinical trials included more than 12,000 women and were done using Gardasil, the quadrivalent formulation, as a control. They showed that, against HPV-6, HPV-11, HPV-16, and HPV-18, Gardasil 9 induced neutralizing antibodies levels comparable to those of Gardasil, which implied equivalent disease-prevention efficacy.[388] Gardasil served as a placebo to assess vaccine efficacy against diseases caused by HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58. Against CIN2+ and all CIN, vaccine efficacy was 96.3% (CI, 79.5% to 99.8%) and 97.7% (CI, 92.2% to 99.6%), respectively. Against vulvar and vaginal diseases, the figures for VIN2/3+ or VaIN2/3+ and all VIN or VaIN were 100% (CI, 71.5% to 100%) and 93.8% (CI, 61.5% to 99.7%), respectively. The efficacy of Gardasil 9 in reducing the number of genital warts or of cervix biopsies was overall 96.9% (CI, 93.6 to 98.6%).[388] Therefore the addition of five strains in Gardasil is expected to broaden the protection not only against cancer but also, and to a greater degree, against the more common CIN1 and CIN2/3, thus offering the prospect of simpler cervical cancer screening guidelines. Full vaccine efficacy is still present for at least 6, 8, and 9 years with Gardasil 9, Gardasil, and Cervarix, respectively.[389–391] A minimal protective antibody threshold has not been established yet.

All the HPV vaccines were originally licensed for a three-dose schedule of immunization. Several studies have shown that a two-dose schedule is as effective and may be advantageous in resource-limited countries.[392–397] It is unknown at this time if beyond 5 years the two-dose schedule will remain effective or if a booster will be necessary.[392]

**TABLE 143.2 Summary of Cervical Cancer Screening Guidelines**

| | |
|---|---|
| **When to Begin Pap Test Screening** | |
| USPSTF, ACS, ACOG | Age 21 years |
| **How Often?** ***Cytology (21- to 65-Year-Olds)*** | |
| USPSTF, ACS, ACOG | Every 3 years, regardless of the cervical cytology technique used |
| ***HPV DNA Co-test*** | |
| *21- to 29-Year-Olds* | |
| USPSTF, ACS, ACOG | No |
| *30- to 65-Year-Olds* | |
| USPSTF | Every 5 years is optional |
| ACS, ACOG | Every 5 years is recommended |
| **When to Discontinue Screening** | |
| USPSTF, ACS, ACOG | At age 65 years |
| | INCLUDE: |
| USPSTF, ACS, ACOG | Women with adequate screening history defined as three consecutive negative cytology results or two consecutive negative HPV DNA co-tests within 10 years of cessation of screening, with the most recent test performed within 5 years |
| | EXCLUDE: |
| ACS | Women age 65 years or older with a history of CIN 2, CIN 3, or adenocarcinoma in situ should continue screening for at least 20 years after spontaneous regression or proper management |
| ACOG | Women with a history of: (a) HIV infection, (b) CIN 2 or higher, (c) immunocompromised, (d) in utero exposure to diethylstilbestrol |
| **Screening After Hysterectomy** | |
| USPSTF, ACS, ACOG | Not necessary if it was a total (uterus + cervix) hysterectomy |
| **Screening Among Those Immunized Against HPV16/18** | |
| USPSTF, ACS, ACOG | No change in the screening guidelines at present |
| **Screening of HIV Seropositive Women CDC/NIH/IDSA** ***Women Younger Than 30 Years*** | |
| | • Start within 1 year of onset of sexual activity or at the time of HIV diagnosis, but not later than age 21 years<br>• Screening is done by cytology alone, not by co-testing<br>• If initial testing is negative, repeat 12 (possibly 6) months later<br>• If the results of three consecutive tests are normal, then screen every 3 years |
| ***Women Aged 30 Years or More*** | |
| | • Start at the age of HIV diagnosis if not started earlier<br>• Screening is done either by cytology or co-testing<br>• If screening is done by ***cytology***, the testing frequency guidelines as the same as for younger women. If cytology shows more than ASC-US, refer for colposcopy. If cytology shows ASC-US, repeat it in 6–12 months. If the result is ASC-US or worse, refer for colposcopy<br>• If screening is done by ***co-testing***:<br>  • Both tests (cytology + HPV) are entirely negative, then repeat in 3 years<br>  • Cytology is negative but HPV is positive (but not for HPV-16/18), then repeat screening in 1 year. If at that time either test is abnormal, refer patient to colposcopy<br>  • Cytology is negative and HPV is positive for types 16 or 18, refer the patient to colposcopy<br>  • Cytology is abnormal for ASC-US and HPV is positive or cytology is abnormal for worse than ASC-US, refer the patient to colposcopy. If the cytology is positive for ASC-US and HPV is negative, repeat cytology in 6–12 months. If the result is ASC-US or worse, refer the patient to colposcopy |

*ACOG,* American College of Obstetrics and Gynecology; *ACS,* American Cancer Society; *ASC-US,* atypical squamous cells of unknown significance; *CDC,* Centers for Disease Control and Prevention; *CIN,* cervical intraepithelial neoplasia; *HIV,* human immunodeficiency virus; *HPV,* human papilloma virus; *IDSA,* Infectious Diseases Society of America; *NIH,* National Institutes of Health; *USPSTF,* US Preventive Services Task Force.

*From American Cancer Society (ACS)[368]: http://onlinelibrary.wiley.com/doi/10.3322/caac.21139/pdf; US Preventive Services Task Force (USPSTF)[369]: https://www.uspreventiveservicestaskforce.org/Page/Document/UpdateSummaryFinal/cervical-cancer-screening; and American College of Obstetrics and Gynecology (ACOG) [370]; Centers for Disease Control and Prevention/National Institutes of Health/Infectious Diseases Society of America (CDC/NIH/IDSA): https://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf.*

The effect of HPV vaccination has already been substantial. Even in the United States, where the vaccine uptake has been poor, the cervicovaginal prevalence of the vaccine HPV types has decreased from 11.5% to 4.3% among females aged 14 to 19 years, within 4 years of the vaccine introduction.[398] In Australia, where vaccine uptake has been high, comparing cervical HPV prevalence in 18- to 24-year-olds before (2005–07) and after (2007–09) the introduction of immunization, the adjusted prevalence ratio has been an impressive 0.07 in immunized women and 0.65 in nonimmunized women, which suggests the existence of herd immunity.[399] In Costa Rica vaccination with HPV-16/18 or placebo of 7466 women led to, 4 years later, a prevalent oral HPV-16/18 infection in 1 subject who received the vaccine and in 15 placebo recipients, thus strengthening the expectation of an ultimate reduction of oropharyngeal cancer incidence.[400] More significant, HPV-associated disease incidence has also decreased after HPV vaccination in many population-based studies conducted both in the United States and abroad. A meta-analysis of 20 vaccine studies has shown a greater than 90% decrease of the relative risk of genital warts in women aged 15 to 19 years in countries with vaccine coverage superior to 50%.[401] This decrease was about 30% in countries with less than 50% vaccine coverage. High vaccine coverage was associated with herd immunity in the older age classes of women but also in all age classes of men. There is now

well-established evidence of a beneficial effect of HPV vaccination on the incidence and prevalence of CIN.[402–405] This gives further assurance that in a few years the effect of vaccination on HPV-associated cancers will be demonstrable beyond the early evidence already observed in Finland.[380a]

The HPV vaccines appear to be very safe (https://www.cdc.gov/vaccinesafety/vaccines/hpv-vaccine.html). Local reactions to immunization are common and include pain, redness, and swelling, with the corresponding rates of 89.9%, 34.0%, and 40.0% for Gardasil 9; 83.5%, 25.6%, and 28.8% for Gardasil; and 92.9%, 44.3%, and 36.5% for Cervarix.[406] These differences reflect mostly the nature and quantities of adjuvants. Systemic adverse reactions of any grade include headache, fatigue, and arthralgia in less than half of the recipients.[406] Vaccine safety has also been monitored extensively through several public and industrial surveillance programs in place in the United States and elsewhere.[407,408] Other than the rare occurrence of allergy and anaphylaxis, there has been no consistent evidence that the HPV vaccines increase the rate of autoimmune disorders, neurologic conditions, or thromboembolic events.[407–413] Syncopes may occur after vaccination, mandating a 15-minute period of observation before discharging the patient, but this appears to be an age-related rather than a vaccine-related complication.[408]

The current (May 2018) HPV vaccination guidelines of the CDC's Advisory Committee on Infection Prevention are presented in Table 143.3 (https://www.cdc.gov/vaccines/vpd/hpv/hcp/recommendations.html). Routine immunization of males and females is recommended at 11 or 12 years (it may be started at age 9 years and should be started this early in children with a history of sexual abuse). Vaccination is recommended in females aged 13 through 26 years and in males aged 13 through 21 years who have not been adequately vaccinated previously.[414–416] The vaccination window should begin at age 9 years but can be extended until age 26 years for gay and bisexual men, MSM, transgender persons, and for immunocompromised persons (including HIV infection) not adequately vaccinated previously. For maximum benefits, adolescents should be immunized before exposure to HPV, but previous infection to one or more HPV types still allows full protection against the other types in the vaccine. Two doses of vaccine (the second dose is to be given 6–12 months after the first) are given to most persons starting the series before their 15th birthday. Adolescents who receive two doses less than 5 months apart will require a third dose. Three doses (0, 1–2, and 6 months) are recommended for teens and young adults who start the series at ages 15 through 26 years and for immunocompromised persons (including HIV infection) ages 9 through 26 years. If the immunization series is interrupted, there is no need to restart; one just completes the series. Persons are adequately vaccinated if they previously received Cervarix, Gardasil, or Gardasil 9, before age 15 years as two doses (at 0, 6–12 months) or three doses (at 0, 1–2, 6 months), or at age 15 years or older in three doses (at 0, 1–2, 6 months). Gardasil 9 is the only vaccine available in the United States at present. It is administered intramuscularly.

**TABLE 143.3 ACIP Recommendations for HPV Immunization**

**HPV Vaccine Recommendations**

- HPV vaccine is routinely recommended for adolescents at age 11 or 12 yr.
- Vaccination is also recommended for females ages 13–26 yr and males ages 13–21 yr who are not adequately vaccinated when they were younger.
- Vaccination is also recommended for gay, bisexual, and other men who have sex with men; transgender persons; and persons with certain immunocompromising conditions ages 22–26 yr who were not adequately vaccinated when they were young.

**HPV Vaccine Safety**

- 9-valent HPV vaccine was studied in more than 15,000 males and females.
- Quadrivalent HPV vaccine was studied in more than 29,000 males and females.
- Bivalent HPV vaccine was studied in more than 30,000 females.
- Each HPV vaccine was found to be safe and effective.

*ACIP,* Advisory Committee on Immunization Practices; *HPV,* human papillomavirus. *From Centers for Disease Control and Prevention (CDC). HPV vaccine information for clinicians. https://www.cdc.gov/hpv/hcp/need-to-know.pdf. Accessed May 22, 2018.*

The contraindications include a severe allergic reaction (e.g., anaphylaxis) to a vaccine component (Gardasil 9 is produced in *Saccharomyces cerevisiae* [baker's yeast]) or after a prior dose of the HPV vaccine. Although the vaccines are contraindicated during pregnancy, both during the clinical trials and the postmarketing surveillance, many women became pregnant, and no excess of congenital malformations or miscarriages has been noted.[417] Gardasil 9 is safe to be administered concomitantly with the other CDC-recommended routine immunizations. HPV vaccination status does not change cervical cancer screening (Pap smear) recommendations.

Cervical cancer screening guidelines are not changed if the woman is vaccinated.

The HPV vaccine is strictly prophylactic and has no impact on the evolution of existing lesions. However, there is evidence that vaccination reduces the recurrence rate of high-grade AIN in males and of HPV-related genital disease in females.[418,419]

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*The complete reference list is available online at Expert Consult.*

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