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144 JC, BK, and Other Polyomaviruses: Progressive Multifocal Leukoencephalopathy (PML)

C. Sabrina Tan and Igor J. Koralnik

SHORT VIEW SUMMARY

JC VIRUS

Definition
- JC virus (JCV) is a ubiquitous human polyomavirus that causes several central nervous system diseases in immunocompromised patients, including progressive multifocal leukoencephalopathy (PML), JCV granule cell neuronopathy, JCV encephalopathy, and JCV meningitis.

Epidemiology
- JCV infects 40% to 86% of the general population worldwide.
- JCV can be detected in the urine of one-third of healthy and immunosuppressed individuals.
- PML can occur in up to 5% of untreated patients with acquired immunodeficiency syndrome.
- Up to 82% of PML patients are infected with human immunodeficiency virus.

Microbiology
- JCV is a member of the Polyomaviridae family, *Orthopolyomavirus* genus.
- It is a double-stranded DNA virus without an envelope.
- After primary infection, JCV remains latent in the kidney tubular epithelial cells.
- Reactivation of JCV causes a lytic infection of oligodendrocytes in the brain, leading to PML.

Diagnosis
- Definitive Diagnosis of PML
  - JCV DNA is detected in cerebrospinal fluid by polymerase chain reaction (PCR) assay, or JCV proteins are detected in brain tissues through histopathologic assessment.
- Possible Diagnosis of PML
  - Magnetic resonance imaging findings and clinical presentation are consistent with PML in the absence of detection of JCV in cerebrospinal fluid.

Therapy
- There are no effective antiviral medications.
- Immune reconstitution can boost host cellular immune response to better control JC viral replication.

Prevention
- Measures should be taken to prevent immunosuppression.

BK VIRUS

Definition
- BK virus (BKV) is a ubiquitous human polyomavirus that causes hemorrhagic cystitis in hematopoietic stem cell transplantation patients and nephropathy in kidney transplantation recipients.

Epidemiology
- BKV infects 82% to 90% of the general population worldwide.
- BKV can be detected in the urine of up to 20% of asymptomatic healthy individuals.
- BKV nephropathy occurs in up to 10% of kidney transplant patients.
- BKV-associated disease can occur in up to 15.9% of allogeneic stem cell transplant recipients.

Microbiology
- BKV is a member of the Polyomaviridae family, *Orthopolyomavirus* genus.
- It is a double-stranded DNA virus without an envelope.
- After primary infection, BKV remains latent in the kidney tubular epithelial cells.
- Reactivation of BKV causes hemorrhagic cystitis and nephropathy.

Diagnosis
- BKV DNA is detected through PCR assay in blood and sustained viruria in urine.
- Cytopathologic changes may be detected in a kidney biopsy specimen.

Therapy
- There is no effective antiviral medication.
- Reduction in immunosuppression can boost host cellular immune response to better control BKV replication.

Prevention
- Early detection of viral reactivation in either urine or blood with PCR assay is an indicator for a preemptive reduction in immunosuppression to help reduce occurrence of renal disease.

The human polyomaviruses, JC virus (JCV) and BK virus (BKV), are ubiquitous in most human populations throughout the world and do not cause disease in immunocompetent individuals. As much as 90% and 86% of the general adult population are seropositive for BKV and JCV, respectively.[1,2] In individuals with immunosuppression, JCV is the etiologic agent of a demyelinating disease of the central nervous system (CNS)—progressive multifocal leukoencephalopathy (PML)[3,4]—whereas BKV causes nephropathy, hemorrhagic cystitis, and ureteral stenosis. PML is an acquired immunodeficiency syndrome (AIDS)–defining opportunistic infection.[5] BKV nephropathy is a cause of allograft loss in kidney transplant recipients. The polyomavirus family has expanded with the discoveries of new viruses and their associated diseases.

VIROLOGY

History

Polyomaviruses are small (45-nm) nonenveloped viruses that are composed of 72 capsomeres with icosahedral symmetry containing a circular double-stranded DNA. The Polyomaviridae family is composed of three genera: two mammalian genera, *Orthopolyomavirus* and *Wukipolyomavirus;* and one avian genus, *Avipolyomavirus*.[6] The polyomaviruses are ubiquitous in nature and are species specific, including humans (JCV, BKV), monkeys (simian virus 40 [SV40]), and mice (murine polyomavirus). In humans, JCV was first isolated from the brain of a patient with PML whose initials were J.C.[7] Similarly, B.K. were the initials of a kidney transplant patient in whom BKV-associated ureteral stenosis was first described.[8] Since 2007, the Polyomaviridae family has expanded to include newly discovered viruses. These were named after the site of discovery—WUPyV (Washington University) and KIPyV (Karolinska Institute)[9,10]; their geographic origins—MWPyV (Malawi)[11] and STLPyV (St. Louis)[12]; the diseases that they cause—MCV (Merkel cell carcinoma)[13] and TSPyV (trichodysplasia spinulosa)[14]; or their order of discovery—HPyV6, HPyV7, HPyV9, HPyV12, and HPyV 13 (human polyomaviruses 6, 7, 9, 12, 13).[15–17]

Epidemiology

Both JCV and BKV have worldwide distribution, including geographically isolated populations with little exposure to other infections.[18,19]

Although seroprevalence for both BKV and JCV increases rapidly with age,[20] primary infection of each virus occurs independently.[21] Acquisition of both viruses through vertical transmission has been documented.[22] After primary infection, BKV and JCV can be detected in renal tubular epithelial cells, where they may remain. Although JC viruria occurs independent of the host's immune status, JC viremia is usually detected only in individuals with immunosuppression. JCV is detected in the urine of 20% to 30% of healthy and immunosuppressed individuals alike, with or without PML.[23,24] However, JCV viremia has been detected in 20% to 40% of individuals who are human immunodeficiency virus (HIV) positive without PML and in 60% to 80% of patients with PML.[25,26] Conversely, BK viruria occurs in 0% to 20% of asymptomatic immunocompetent individuals,[1] but viral shedding in the urine is higher in individuals with immunosuppression (10%–60%),[27–29] correlating to the degrees of immunosuppression.[30] Specifically, in HIV-positive patients, BK viral load in the urine increases with decreased $CD4^+$ T-cell counts.[31,32] Although BKV is usually not detected in the peripheral blood of patients with either immunocompetence or immunosuppression, the detection of BKV DNA in the plasma of renal transplant patients is indicative of an increased risk of development of BKV-induced nephropathy.[27,33] In a cohort analysis of 491 patients undergoing hematopoietic stem cell transplant (HSCT), investigators reported that BKV-associated disease occurred in 15.9% and was associated with significant and prolonged morbidity.[34] Patients who underwent reduced-intensity, double umbilical cord blood cell transplantation had BK viremia frequently detected (15/27) within 100 days of transplantation.[35]

Genome

Polyomaviruses have a circular double-stranded genomic DNA of approximately 5 kb. The genomes of BKV and JCV have been used extensively in human population migration studies because of their extremely conserved coding regions. JCV and BKV share approximately 70% genome homology,[36] and all polyomaviruses maintain the feature of coding DNA from both strands. The early proteins, large T and small t, are transcribed counterclockwise from one strand, whereas the late proteins, VP1, VP2, VP3, and agnoprotein, are transcribed clockwise from the opposite strand (Fig. 144.1). The genome can be divided into three distinct areas: (1) the early genes region, which includes the large T and small t antigens, regulatory proteins responsible for viral transformation, replication, and regulation of gene expression; (2) the late genes region, which encodes the capsid proteins VP1, VP2, and VP3 and the agnoprotein; and (3) the noncoding regulatory region. The agnoprotein is important both in regulation of JCV transcription and translation and in dysregulation of host cell cycle and DNA repairs.[37,38] Similar to the origin of DNA replication, the noncoding regulatory region also contains several binding sites for nuclear factors, which play important roles in JCV transcription. Deletions, insertions, and rearrangements in the regulatory region are associated with tissue tropism and virulence. The archetype regulatory region is mainly found in JCV isolated from kidney cells and consequently from urine. It is thought to be the type from which all other JCV regulatory region sequences have evolved. The rearranged type of regulatory region of JCV is principally detected in the brain and cerebrospinal fluid (CSF) of patients with PML.[39] Whereas the rearranged type of regulatory region is found in the peripheral blood of patients with PML, a mixture of both types is present in the bone marrow.[25,40] Which type of regulatory region is associated with primary infection is not known.

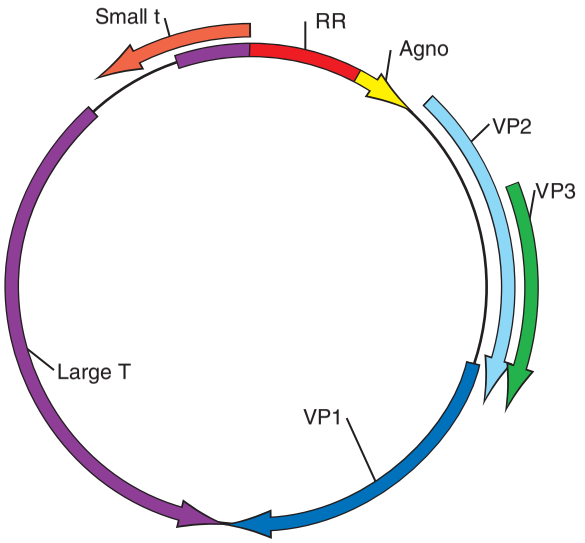

FIG. 144.1 Polyomavirus genomic map (~5 kb). *RR,* Regulatory region. *(Courtesy Dr. Yiping Chen.)*

Receptors and Cell Entry

JC Virus

Data suggest that *N*-linked glycoprotein containing an α(2,6)-linked sialic acid may act as a JCV receptor.[41] In addition, evidence from cultured cell lines shows that JCV uses the serotonergic 5-HT2A receptors for cell entry.[42] Pharmacologic blockade of the serotonergic receptors restricts JCV infection in vitro. The 5-HT2A receptor is present on glial cells and astrocytes and on B lymphocytes, platelets, and kidney epithelial cells. JCV also uses clathrin-coated pits on cell surfaces after binding and adsorption, mediated by the capsid protein VP1. Cell entry occurs via endocytosis and results in fusion of virus-carrying vesicle with the nuclear membrane.[43]

BK Virus

BKV uses an *N*-linked glycoprotein containing an α(2,3)-linked sialic acid as a receptor. Entry into the cell occurs via caveolae-mediated endocytosis.[44]

New Human Polyomaviruses

Seroprevalences for the newer human polyomaviruses range from 25% to 90%.[15,45–48] The KIPyV and WUPyV polyomaviruses were first isolated from respiratory samples with high-throughput screens.[10,49] Subsequently, molecular evidence indicated a worldwide distribution for both viruses in the respiratory secretions of children, with incidence rates as high as 2.5% in Australia[50] for KIPyV and 7% in South Korea[51] for WUPyV. A study analyzing nasopharyngeal aspirates in the United Kingdom showed a bimodal age distribution of KIPyV and detection of the WUPyV in samples from patients younger than 15 years old.[52] Although both WUPyV and KIPyV could be colonizers of the respiratory tracts, KIPyV was associated with respiratory symptoms in transplant recipients.[53] HPyV6, HPyV7, MWPyV, and STLPyV were also all recovered from nonsterile sites such as skin and stool. Therefore the proof of their existence as bona fide infectious agents remains to be established. Like KIPyV, these viruses could be part of the human microbiota that causes problems only in immunosuppressed patients. For example, HPyV7 was found to be the cause of a pruritic rash in a lung transplant recipient.[54] Lastly, the detection of HPyV9 in normally sterile blood distinguished this virus as a possible human pathogen.

The Merkel cell polyomavirus (MCV), discovered in 2008, and trichodysplasia spinulosa polyomavirus (TSPyV), discovered in 2010, are both associated with human skin diseases. MCV can be detected in up to 80% of tissues with Merkel cell carcinoma, an aggressive and deadly neuroectodermal tumor that principally affects patients with immunosuppression.[13] Molecular studies indicate that MCV shares a similar genome with the other human polyomaviruses and that integration of MCV into the tumor genome most likely takes place before clonal expansion of tumor cells. MCV is most similar to the African green monkey lymphotropic polyomavirus. TSPyV viral particles were detected with electron microscopy on a hair follicle from a heart transplant patient with trichodysplasia spinulosa, a rare skin disease affecting immunocompromised patients.[14]

Interesting to note, unlike JCV and BKV, these newer human polyomaviruses do not contain a gene that encodes the agnoprotein.

The agnoprotein was initially named because its function was unknown. However, this protein appears to have important regulatory functions of both polyomaviruses and host cells. The significance of the agnoprotein deletion in these polyomaviruses remains to be determined.

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY AND OTHER JC VIRUS–ASSOCIATED SYNDROMES

Epidemiology

PML was initially described in 1958.[55] The disease was estimated to occur in 0.07% of individuals with hematologic malignant diseases,[56] such as leukemia and lymphoma, or rarely in patients with solid-organ cancer or organ transplants. Since the 1980s, in the era of HIV infection, PML has been recognized as a major opportunistic infection that affects up to 5% of patients with AIDS.[5] Despite the advent of combined antiretroviral therapy (ART), PML still occurs in 0.6 to 1.3 per 1000 HIV-positive person-years.[4] Currently, 82% of patients with PML have AIDS, 8.4% have hematologic malignant diseases, 2.8% are solid-organ transplant recipients, 0.95% have chronic inflammatory diseases, and 6% have no defined risk factor for PML.[4] Indeed, 38 cases of PML have been reported in HIV-negative individuals with minimal or occult immunosuppression.[57] In HIV-negative individuals, the incidence rate of PML per 100,000 person-years is 0.3 in the general population, 1.0 in patients with rheumatoid arthritis, 2.4 in those with systemic lupus erythematosus, 10.8 in those with autoimmune vasculitis, 8.3 in those with non-Hodgkin lymphoma and chronic lymphocytic leukemia, and 35.4 in bone marrow transplant recipients.[58] Lastly, the newest group of patients with PML has emerged among patients treated with immunomodulatory medications for malignant diseases or autoimmune diseases, such as patients treated with natalizumab and dimethyl fumarate for multiple sclerosis (MS) or Crohn disease,[59–63] rituximab for lymphoma[64,65] or lupus,[66] and efalizumab or fumaric acid for psoriasis.[67,68]

Natalizumab-treated MS patients are at increased risk of developing PML. As of November 30, 2017, 756 cases of natalizumab-related PML had been documented worldwide. Risk of PML increases in patients with longer natalizumab treatment duration, prior use of other immunosuppressants, and seropositivity for JCV. The incidence of natalizumab-associated PML is highest after 49 to 72 months of treatment in JCV-seropositive patients with a prior history of immunosuppressive treatment, where it reached 1.3%.[69]

Pathogenesis

Primary infection with JCV is asymptomatic, after which the virus becomes clinically latent. JCV DNA is detected with polymerase chain reaction (PCR) assay in the tonsillar tissues of 39% of healthy individuals, which suggests a possible oral or respiratory route of infection.[70,71] Detection of JCV in urine of healthy adults indicates that the kidney is a site of latency. Immunohistochemical staining shows viral proteins in the kidney tubular epithelial cells. Detection of JCV DNA in the bone marrow[72] and brain[73] of patients without PML suggests that these are also possible additional sites of latency.

In patients with PML, JCV infects oligodendrocytes and astrocytes in the brain and rarely the spinal cord, which causes a lytic infection that results in the destruction of the myelin sheath. Pathologic examinations of PML lesions show extensive demyelination of the affected areas. Because of the presence of oligodendrocytes and astrocytes in the hemispheric cortex, demyelination can also occur within the cortical gray matter.

Clinical Manifestations

Classic Progressive Multifocal Leukoencephalopathy

Because PML causes multifocal demyelination of the white matter of the CNS, the resulting neurologic deficits correspond to the location of the lesions. Although initial symptoms of PML can vary greatly, depending on the affected anatomic brain region, the predominant symptoms include coordination difficulties, gait imbalance, cognitive dysfunction, visual problems, and limb paresis. The optic nerves and the spinal cord of the CNS are usually spared, but incidental postmortem findings of PML lesions have been discovered in the spinal cord of a patient who was HIV positive with hemispheric PML.[74] Furthermore, although PML lesions are generally located in the white matter, seizures, which are usually considered to be of cortical origin, can occur in up to 44% of PML survivors and are associated with juxtacortical demyelinated lesions and with hyperintense cortical signal (HCS) demonstrated on T1-weighted magnetic resonance images. The histologic correlate of HCS is leukocortical encephalitis, which recapitulates the triad of PML (demyelination, astrogliosis, and infiltration with phagocytic macrophages, albeit in the cortical gray matter) (Table 144.1).[75–77]

Progressive Multifocal Leukoencephalopathy–Immune Reconstitution Inflammatory Syndrome

Inflammatory PML has been frequently detected when the immune response is rapidly restored with ART in patients with HIV. Patients usually have an increasing $CD4^+$ cell count and a decreasing plasma HIV viral load. Unlike classic PML, up to 57% of the PML–immune reconstitution inflammatory syndrome (IRIS) lesions may display contrast enhancement with magnetic resonance imaging (MRI),[4,78] which indicates a breakdown of the blood-brain barrier, along with worsening of the initial presenting symptoms. Conditions may stabilize after the initial worsening of symptoms, but fatal outcome has been reported.[79,80] Magnetic resonance spectroscopy (MRS) helped define a unique metabolic pattern in PML lesions that showed an increased ratio of lipids to creatine consistent with inflammation. Combined with the presence of contrast enhancement in PML lesions, this finding yielded a 79%

TABLE 144.1 JC Virus–Associated Diseases

CLINICAL PRESENTATION	CLASSIC PML	PML-IRIS	JCV GCN	JCV E	JCV MENINGITIS
Onset	Subacute	Immune recovery	Chronic	Subacute	Chronic
Magnetic resonance imaging	Asymmetrical, well-demarcated, nonenhancing subcortical white matter lesions; hyperintense on T2-weighted and FLAIR images; hypointense on T1-weighted images	Contrast-enhancing lesions and possible mass effect	Cerebellar atrophy	Cortical lesions	Enlarged ventricles
Neurologic symptoms	Based on location	Based on location and inflammation	Cerebellar syndrome	Encephalopathy	Headache
Histology	Demyelinating lesions often at gray-white matter junction; JCV detected in enlarged oligodendrocytes; bizarre astrocytes; presence of $CD8^+$ T cells near JCV-infected cells	Demyelination similar to classic PML but with marked inflammatory infiltrates	Focal areas of cell loss in granule cell layer of cerebellum; JCV detected in enlarged granule cell neurons	Focal areas of cell loss in hemispheric cortex; JCV detected in enlarged pyramidal neurons	Productive infection of meningeal and choroid plexus cells

E, Encephalopathy; *FLAIR,* fluid-attenuated inversion recovery; *GCN,* granule cell neuropathy; *IRIS,* immune reconstitution inflammatory syndrome; *JCV,* JC virus; *PML,* progressive multifocal leukoencephalopathy.

probability of IRIS compared with 13% in the absence of these criteria.[81]

In autopsy samples of HIV-positive patients with PML-IRIS, mainly $CD8^+$ lymphocytes were detected in the lesions, along with a paucity of $CD4^+$ lymphocytes.[80,82,83] The reported time at onset of PML-IRIS ranged from 4 to 108 weeks after start of ART.[79,80] In some patients, ART was started as treatment for PML[79,80,82–86]; in others, PML was diagnosed at the time of IRIS presentation.[79,87]

PML-IRIS has also been reported in HIV-negative patients, such as in natalizumab-treated MS patients after plasma exchange to remove natalizumab.[88]

JC Virus Granule Cell Neuronopathy

Demyelination of white matter in the cerebellum is well described in patients with PML. In addition to oligodendrocytes and astrocytes, JCV can also infect and destroy cerebellar granule cell neurons. This neuronal infection can result in a novel syndrome characterized by cerebellar atrophy, gait ataxia, and incoordination, without associated demyelination.[89] This novel syndrome, distinct from PML, is called JCV granule cell neuronopathy (GCN).[90,91] JCV GCN appears to be caused by a JCV variant harboring a 10-bp deletion in the carboxyl terminus of the *VP1* gene.[92] A histologic survey indicated that infection of granule cell neurons may be found in up to half of patients with PML.[93] This finding was confirmed in other JCV GCN isolates from various geographic origins, and other mutations occurring in this area of the *VP1* gene were characterized, strengthening its association with infection of GCN.[4,94] More recently, JCV GCN has also been diagnosed in natalizumab-treated MS patients.[95,96]

JC Virus Encephalopathy

JCV can also infect hemispheric cortical pyramidal neurons. The clinical presentation differs from both classic PML and JCV GCN. Brain lesions are initially restricted to the gray matter at MRI, and the patient presents with a global cognitive decline and aphasia, rather than with focal neurologic deficits such as sensory or motor dysfunction.[97] The JCV strain isolated from cortical samples had a unique deletion in the agnoprotein gene.[4,98]

This deletion impairs VP1 production and virion expression, which may explain a restrictive infection of JCV in cortical pyramidal neurons.[99,100]

JC Virus Meningitis

Although JCV is not routinely tested for in the CSF of patients with meningeal symptoms, several studies have documented JCV as the only pathogen present in the CSF of patients with typical meningitis symptoms, including neck stiffness and diplopia.[101–103] Whether these cases result from JCV primary infection or reactivation is unclear. A case report of JCV meningitis in an HIV-negative patient had reported productive JCV infection of meningeal and choroid plexus cells with limited infiltration of the cerebral parenchyma.[104,105] Because JCV PCR is not routinely performed in the CSF of patients with meningitis who do not have CNS lesions, the exact incidence of JCV meningitis is unknown.

Diagnosis

The diagnosis of PML can be established through either histopathologic or clinical pathways. Whereas the first requires demonstration of JCV infection in the brain, the latter is based on clinical and radiologic findings consistent with PML and not better explained by other disorders, coupled with demonstration of JCV DNA by PCR in CSF. Diagnostic classification includes certain, probable, possible, and not PML.[106]

Because PML remains a relatively rare disease, it is often misdiagnosed, with a median time from initial symptoms to diagnosis of 74 days.[107]

Imaging

Radiographically, PML brain lesions typically appear as multiple white matter lesions, usually sparing the cortex, that do not conform to particular vascular territories. No associated mass effect or contrast enhancement is generally seen. Both computed tomography (CT) and MRI can be used in the diagnosis of PML. Lesions appear in the white matter as hypodense or patchy on CT scans; at MRI, they are areas of hyperintense (bright) signal on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images and show as hypointense (dark) signal on T1-weighted images. MRI is more sensitive than CT and is the imaging method of choice for diagnosis of PML. As the name *progressive multifocal leukoencephalopathy* suggests, multiple lesions are usually present; they are often located in the subcortical white matter or in the cerebellar peduncles.[108,109] Lesions may also be found in gray matter structures, such as the basal ganglia or thalamus, which also contain myelinated fibers. Furthermore, HCS can also be seen overlying white matter lesions of PML.[77] In addition, PML may have atypical presentations, including unifocal and miliary patterns.[110,111] Although features of MRI appearance are not linked to survival time, the rare detection of mass effect predicts poor prognosis.[112] Lastly, atypical PML lesions, including ones with contrast enhancement, are seen in natalizumab-associated PML[113] and in inflammatory PML that results from IRIS (Fig. 144.2).[79]

Brain Biopsy

Brain biopsy is the gold standard for the diagnosis of PML. This test has a sensitivity that ranges from 64% to 96% and a specificity of 100%. Histologic examination shows demyelinated areas, with reactive gliosis and enlarged and bizarre astrocytes, and macrophages that contain phagocytosed myelin and cellular debris. These lesions are located in both cortical and subcortical regions of the brain.[114] JCV can be detected in infected oligodendrocytes with enlarged amphophilic nuclei located at the periphery of the lesions. Intraparenchymal and perivascular infiltrates by $CD8^+$ T cells are usually present in PML-IRIS (Fig. 144.3).[80]

Cerebrospinal Fluid Polymerase Chain Reaction Assay

Brain biopsy may not be feasible in some patients, depending on the location of the lesions and the disability of the patient. In addition, biopsy carries an inherent morbidity and mortality risk. Therefore CSF examination is more commonly used as an alternative method of diagnosis. Patients with PML generally have a nonspecific CSF profile, including mild pleocytosis, with slightly elevated protein and normal glucose levels. The detection of JCV DNA with PCR assay of CSF had a sensitivity of 72% to 92% and a specificity of 92% to 100%[115] before extensive availability of ART. However, in the post-ART era, the sensitivity of PCR detection of CSF has decreased significantly to 58% with no changes in the specificity in patients with HIV presenting with PML while on ART.[116]

Prognosis

PML is a fatal disease in most cases. Before the ART era, only 10% of patients who were HIV positive with PML lived longer than a year after diagnosis.[117,118] After the extensive use of ART, the 1-year survival rate has increased to 50%.[118] A number of studies have delineated several prognostic markers, which include patients who present with a lower JCV CSF burden,[119] detectable JCV-specific immune response in blood and CSF,[120–123] and development of inflammatory immune response in the CNS.[124,125] Because positive JCV serology is detected in most individuals, including those with PML, the humoral immune response alone is not able to prevent viral reactivation. However, cellular immune response to JCV has been detected in patients with PML, and a strong response is associated with better prognosis. Studies have documented the presence of cytotoxic T lymphocytes (CTLs) that recognize two human leukocyte antigen (HLA) A*0201–restricted epitopes of the major capsid protein VP1 in patients with PML who survived more than 1 year.[121,126–128] Presence of JCV-specific CTLs was associated with an increase in 1-year survival of PML from 46% to 73%.[129] In a study of 66 patients, PML survivors were 22.2 times more likely to have both $CD4^+$ and $CD8^+$ T-cell responses against JCV early after disease onset than PML progressors.[130] Furthermore, elevation of myoinositol, a marker of inflammation detected with MRS in PML lesions, was associated with a better prognosis.[125,131] Finally, hyperperfusion within PML lesions, measured with arterial spin labeling MRI, was associated with a worse prognosis.[132]

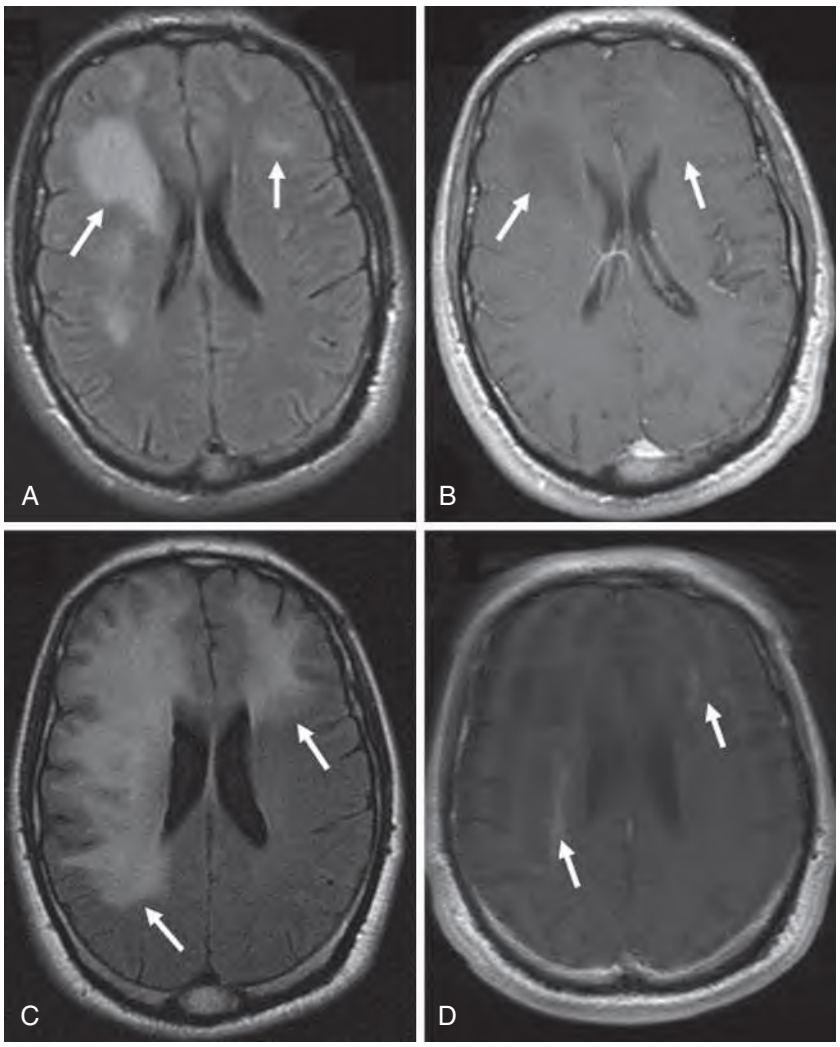

FIG. 144.2 Magnetic resonance images of patient with human immunodeficiency virus infection with classic progressive multifocal leukoencephalopathy at presentation. (A) Fluid-attenuated inversion recovery (FLAIR) image shows lesions in right and left frontal lobes *(arrows).* (B) No enhancement is evident after gadolinium injection on a T1-weighted image *(arrows).* (C) FLAIR image after antiretroviral therapy shows progressive multifocal leukoencephalopathy–immune reconstitution inflammatory syndrome displaying progression of lesions in both hemispheres *(arrows).* (D) Peripheral enhancement can be seen in T1-weighted image after gadolinium enhancement *(arrows).*

Therapy

No specific treatment exists for PML. However, in patients infected with HIV, initiation or optimization of ART has been associated with an increase in PML survival rate at 1 year from 10% to 50%.[118] For patients who are HIV negative, improvement of the immune status through reduction of immunosuppression may allow for the adaptive immune system to take control of the infection. Because a strong cellular immunity against JCV has been associated with a better clinical outcome, immunotherapies aimed at boosting this immune response may become a treatment option for PML.[133] Immune reconstitution after allogeneic HSCT has been associated with selective control of JCV reactivation.[134] Cytarabine has shown an in vitro effect in controlling JCV replication and multiplication.[135] One randomized controlled clinical trial with patients who were HIV positive with PML did not show efficacy,[136] but one small retrospective study did show stabilization after intravenous treatment with cytarabine[137] in 7 of 19 HIV-negative patients (37%) with PML who had leukemia or lymphoma. Cidofovir is another potent antiviral agent that has shown in vitro activities against murine polyomavirus and SV40, but it has not been tested against JCV or BKV. However, multiple studies in different centers with cidofovir in patients both with and without HIV have not shown a significant effect in changing the disease course.[118,138–140] Lastly, because in vitro studies revealed that JCV infects cells via the serotonin receptor 5-HT2A, mirtazapine, a serotonin receptor blocker, has been considered as a potential candidate for containment of JCV infection.[42] However, clinical improvement of PML with mirtazapine remains anecdotal so far.[141] A large pharmacologic screening study suggested that mefloquine, an antimalarial medication, has the ability to inhibit JCV replication in cell culture.[142] A multicenter study examining the role of mefloquine in the treatment of PML was terminated ahead of schedule because of lack of efficacy.[143]

Immunotherapy against JCV is a potential future option. This includes administration of interleukin 7 (IL-7) which boosted both $CD4^+$ and $CD8^+$ T-cell counts in a patient with idiopathic lymphocytopenia who survived PML.[144]

Another option is adoptive transfer therapy, in which virus-specific T cells are stimulated either from patient's own lymphocytes or from a matching donor's lymphocytes. Broadly neutralizing antibodies against multiple JCV VP1 capsid mutations could potentially be infused as therapy.[144a] JCV-specific vaccine against a specific strain was given to a PML patient and induced cognate neutralizing antibodies.[145]

In treatment of patients with inflammatory PML, in whom brain swelling and mass effect can be present, a short course of corticosteroids including prednisone, dexamethasone, and methylprednisolone may be used sparingly for mitigation of the inflammation surrounding the

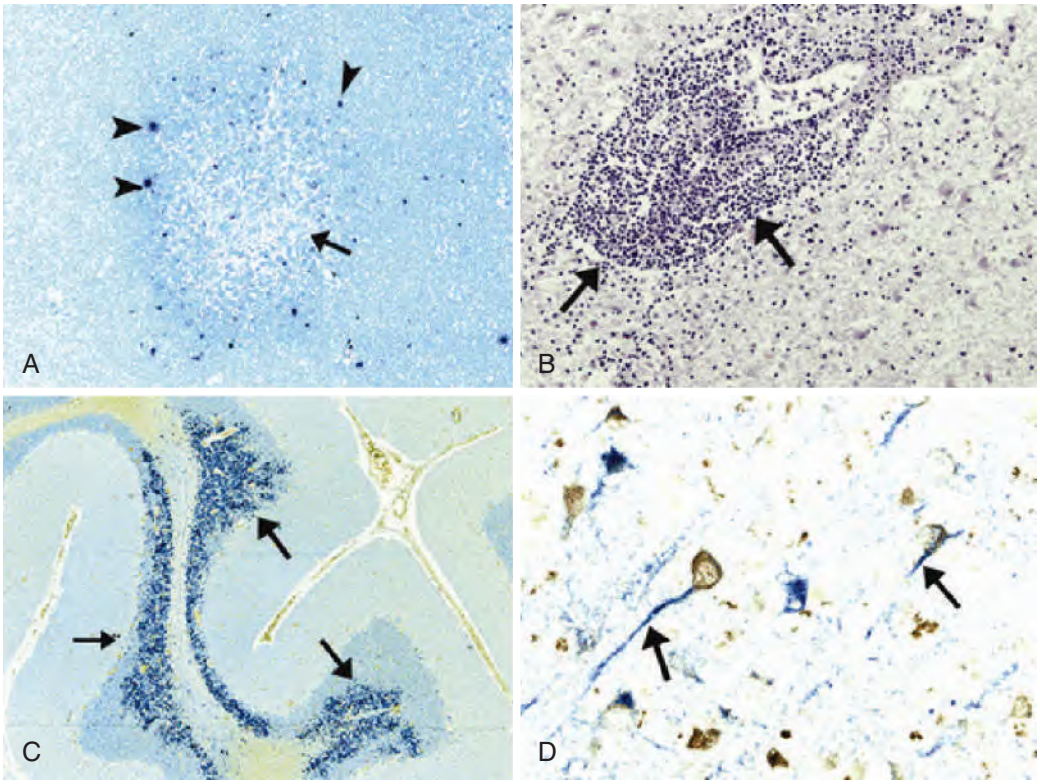

FIG. 144.3 Histologic features of JC virus (JCV) infection in the central nervous system. (A) Classic progressive multifocal leukoencephalopathy (PML), with demyelinating lesion in white matter *(arrow)* surrounded by multiple JCV-infected glial cells *(dark blue, arrowheads).* (B) PML–immune response inflammatory syndrome, with marked lymphocytic perivascular infiltrates *(arrows).* (C) JCV granule cell neuronopathy in a patient with human immunodeficiency virus infection with focal JCV infection of granule cell neurons *(dark blue, arrows).* (D) JCV encephalopathy with hemispheric cortical neurons *(brown)* infected with JCV *(blue, arrows). (A, C, and D courtesy Dr. Christian Wühtrich; B courtesy Dr. Françoise Gray.)*

lesion, especially with a risk for brain herniation. However, the use of these medications in PML-IRIS cases remains a matter of debate.[146,147] In patients with HIV, no evidence exists that interruption of ART during corticosteroid treatment is beneficial. In published case studies, most clinicians chose to continue ART after the diagnosis of PML-IRIS. Short-term interruption of ART can be used temporarily to reduce the uncontrolled immune reaction. However, clinicians need to be aware that disruptions in ART have been associated with higher HIV viral load rebound and lower CD4 counts.[148,149] Furthermore, whether a recurrence of IRIS may develop when ART is restarted is also unclear. Corticosteroids, including dexamethasone, prednisone, and hydrocortisone, have been used anecdotally for their antiinflammatory and immunosuppressive effects.[150] However, no clear guidelines exist for corticosteroid treatments of patients with PML-IRIS. Therefore these medications should be used only in cases with clear clinical or radiologic worsening attributable to IRIS, including life-threatening situations associated with cerebral edema and impending brain herniation.

NEPHROPATHY AND OTHER BK VIRUS–ASSOCIATED DISEASES

Epidemiology

Although BKV-induced nephropathy rarely occurs in a native kidney,[151,152] the prevalence rate of this condition in kidney transplant recipients ranges from 1% to 10%.[153,154] Diagnosis is made an average of 44 weeks after transplantation, with a peak around 24 weeks.[27] With the use of potent immunosuppressive medications, such as tacrolimus and mycophenolate, a trend has been seen toward increasing prevalence of BKV-induced nephropathy in renal transplant patients.[155,156] Incidences of BK viremia and viruria decrease significantly 1 year after transplantation, diminishing the risk for BKV reactivation.[157] In addition, the incidence rate of BKV-induced ureteral stenosis in renal transplant patients is estimated to be 3%.[158] Lastly, hemorrhagic cystitis is the most prevalent BKV-associated complication and occurs in 10% to 25% of bone marrow transplant recipients.[159,160]

Pathogenesis

BKV primary infection occurs in childhood and is asymptomatic. Thereafter, the virus remains latent in the kidney. Most cases of asymptomatic BK viruria are not associated with nephropathy or hemorrhagic cystitis. Therefore BKV pathogenesis is the result of several factors, including host predisposition, target organ damage, and immunosuppression. During the initial posttransplant period, severe therapeutic immunosuppressive conditioning regimens are a trigger for reactivation of BKV in renal cells. Such reactivation may lead to productive infection and lysis of kidney tubular epithelial cells. Although the ability of the host to mount BKV-specific immunity can be limited by immunosuppressive medications, the resulting cell lysis and necrosis mediated by the immune cells may trigger further renal dysfunction. In patients with renal transplantation, BK nephropathy is associated with risk factors including age, sex, race, BK serostatus of the recipients, ABO incompatibility, and types of immunosuppression used.[161–164] In HSCT patients, BKV seropositivity, older age, high anti-BKV immunoglobulin G (IgG) levels before transplant, and lower total lymphocyte count have been associated with increased risk for BKV reactivation.[165,166] Furthermore, in the late engraftment period, the recovery of immune function may produce additional injury from inflammation caused by immune reconstitution, resulting in hemorrhagic cystitis.[167] Lastly, alloimmune dysregulation may play an important role because BKV-induced hemorrhagic cystitis is rarely detected in autologous transplant recipients who received similar conditioning regimens.[168]

Clinical Manifestations

Nephropathy

BKV has a tropism for cells of the genitourinary tract. Infection with BKV in patients with immunocompromise can result in asymptomatic

hematuria, both hemorrhagic and nonhemorrhagic cystitis, and ureteral stenosis. Furthermore, BKV can induce interstitial nephritis in patients with HIV or renal transplantation. However, in patients with a renal allograft, BKV-induced renal tubular epithelial infection results in nephropathy, which is not associated with any specific immunosuppressive medication. BK viruria and viremia usually precede nephropathy. The factors associated with detection of BKV in urine and blood include high donor BK antibody titer and, possibly, absence of the HLA-C7 class I allele in both donor and recipient.[169] In addition, a higher risk for development of BKV nephropathy is associated with older age, male gender, comorbidity of diabetes mellitus, white race, and placement of ureteral stent.[154,170] Onset of BKV nephropathy after transplant ranges from 6 days to 5 years, with a mean time of 10 to 13 months.[171,172] Clinical manifestations of BKV nephropathy are similar to that of graft rejection, with slowly increasing serum creatinine levels without symptoms. Hematuria and fever can be detected in some patients.[173,174] Laboratory findings suggest renal insufficiency and urinary abnormalities. A rise in quantitative BKV serology can be seen with reactivation of the virus; however, this increase does not prevent progression of disease.[169]

Ureteral Stenosis

Renal transplant patients with ureteral stenosis do not usually present with pain or discomfort because the transplanted kidney is not innervated. However, patients can present with urinary obstruction and laboratory findings of elevated serum creatinine levels.

Hemorrhagic Cystitis

BK viruria in HSCT patients is associated with increased mortality.[175] Because of BKV tropism for renal urinary tract cells, up to 50% of HSCT patients develop BK viruria within 2 months of transplant,[36,159] usually after engraftment.[176] The rate of BK viruria is no different between allogeneic versus autologous grafts.,[177] but only a portion of those with viruria develop hemorrhagic cystitis (10%–25%), ureteral stenosis, and interstitial nephritis. Furthermore, most cases of hemorrhagic cystitis occur in allogeneic HSCT recipients who also have graft-versus-host disease,[168] which indicates that immune reconstitution is part of the pathogenesis. Myeloablative conditioning regimen and graft-versus-host diseases have been identified as risk factors that predisposeHSCT patients to BK viruria and hemorrhagic cystitis.[178–180] Diagnosis of BKV-induced hemorrhagic cystitis is considered when postengraftment HSCT patients develop hematuria, dysuria, urgency, frequency, or suprapubic pain. Severe bleeding and clot formation can lead to complications, including urinary tract obstruction and renal failure.

Infections Outside the Renal System

BKV has been reported to disseminate to extrarenal organs. Multiple case reports have described encephalitis in immunosuppressed patients with detection of BKV in their CSF and brain tissues with PCR assay.[181–185] Furthermore, BKV-associated pneumonia and pneumonitis are also reported in HSCT patients after hemorrhagic cystitis.[186–188] Detection of BKV in the urine has also been associated with genitourinary tumors.[189]

Diagnosis

Urine Polymerase Chain Reaction Assay

In the appropriate clinical setting, detection of BK viruria and renal insufficiency is diagnostic of BKV-induced nephropathy. Detection of BKV in urine often precedes viral detection in the blood, and sustained viruria, defined as detection in two or more consecutive urine samples, can be 100% sensitive and 94% specific for BK viremia.[190] Urine cytologic evaluation that reveals decoy cells (enlarged nucleus with a single large basophilic intranuclear inclusion) is an indication of viral infection, but is not specific to BKV infection because these cells are also associated with CMV and adenovirus infections.[191,192] Although detection of BKV DNA with PCR assay in the urine of patients with hemorrhagic cystitis is sensitive, it is nonspecific because asymptomatic BK viruria is common. However, the presence of an increased BK viral load in urine along with hematuria supports the diagnosis.

Renal Biopsy

Renal biopsy is essential in the diagnosis of nephropathy. Histologic examination of renal biopsy shows viral replication in the tubular epithelium cells with large intranuclear inclusions and cell detachment (Fig. 144.4).[193] These cytopathic changes are initially localized to the medulla and distal tubules, with progression to the proximal tubules. However, renal biopsy is associated with a false-negative rate of up to 30% because of the focal nature of the disease.

Plasma Polymerase Chain Reaction Assay

BKV DNA can be detected in the plasma of kidney transplant recipients.[194] However, this test is more useful in ruling out BKV nephropathy than in diagnosing it. BKV PCR in blood has a negative predictive value of 100% but a positive predictive value of only 50% for BKV nephropathy.[36] One study, however, indicated that plasma BK viral load above 10^4 copies/mL has a sensitivity and specificity of 93% in prediction of histologic manifestations of nephropathy.[195] Unlike in nephropathy, BKV is usually not detected in the blood of patients with hemorrhagic cystitis or ureteral stenosis. However, one study found that BK viremia in HSCT patients is an independent predictor for development of post-HSCT renal impairment.[196]

Prognosis

BKV-induced nephropathy is associated with irreversible graft failure in 1% to 10% of cases.[70] Histologic findings of mild viral cytopathic changes with minimal inflammatory infiltrates or fibrosis correlated with better prognosis for allograft outcome.[195] Studies have shown[197] decreased incidence of nephropathy with regular and early screening for BK viruria, viremia, and urine cytologic detection of virus-infected decoy cells and subsequent reduction of immunosuppressive agents.[33,198–207] Poor outcome from BKV–induced nephropathy is influenced by higher BKV serum viral load, immunosuppression with tacrolimus, and delayed diagnosis.[208] In post–renal transplant patients with BKV reactivation, those patients with BKV-specific cellular responses curtail viral replication within a median duration of 1 month without other interventions.[209] Furthermore, in patients who developed BKV nephropathy, a robust BKV-specific cellular immune response correlates with decreased viruria and viremia, whereas the humoral immune response correlated with decreased viremia only.[210]

Prevention and Therapy

Nephropathy

Systematic screening for BKV reactivation in post–renal transplant recipients is effective in preventing nephropathy. Detection of BKV in blood is usually followed by reduction of immunosuppressants to revive adaptive immune responses against BKV.[211] Screening for BKV in urine can detect reactivation earlier because viruria almost always occurs before viremia. However, not all patient with viruria will progress to develop viremia.

Treatment of BKV-induced nephropathy is reduction of immunosuppression. Multiple medications, including cidofovir, leflunomide, quinolones, and intravenous immune globulin (IVIG), have shown varying success in small clinical studies.[212–216] However, patients in these studies also had concomitant decrease of immunosuppressive medications. Because no specific antiviral therapy exists for BKV, reduction of immunosuppression is the therapy of choice[200] and has to be balanced with the increase in risk for graft rejection. The clearance of BKV from plasma is a surrogate marker for resolution of renal tissue pathologic conditions. Fluoroquinolones, such as ciprofloxacin, can inhibit BKV replication in vitro by inhibiting BKV-encoded DNA gyrase.[217] Two small retrospective studies demonstrated decreased BKV infection after renal transplantation in the group with exposure to fluoroquinolones.[218,219] However, two recently conducted randomized, placebo-controlled studies showed that 3 months of treatment with levofloxacin did not reduce the rate of BK viruria, but did result in increased resistance to levofloxacin in bacterial isolates.[220,221] Antibody-based treatment of BKV was based on the finding that neutralizing antibodies against BKV are specific to viral capsid sequences.[222,223] Several case series have demonstrated a potential role for IVIG in

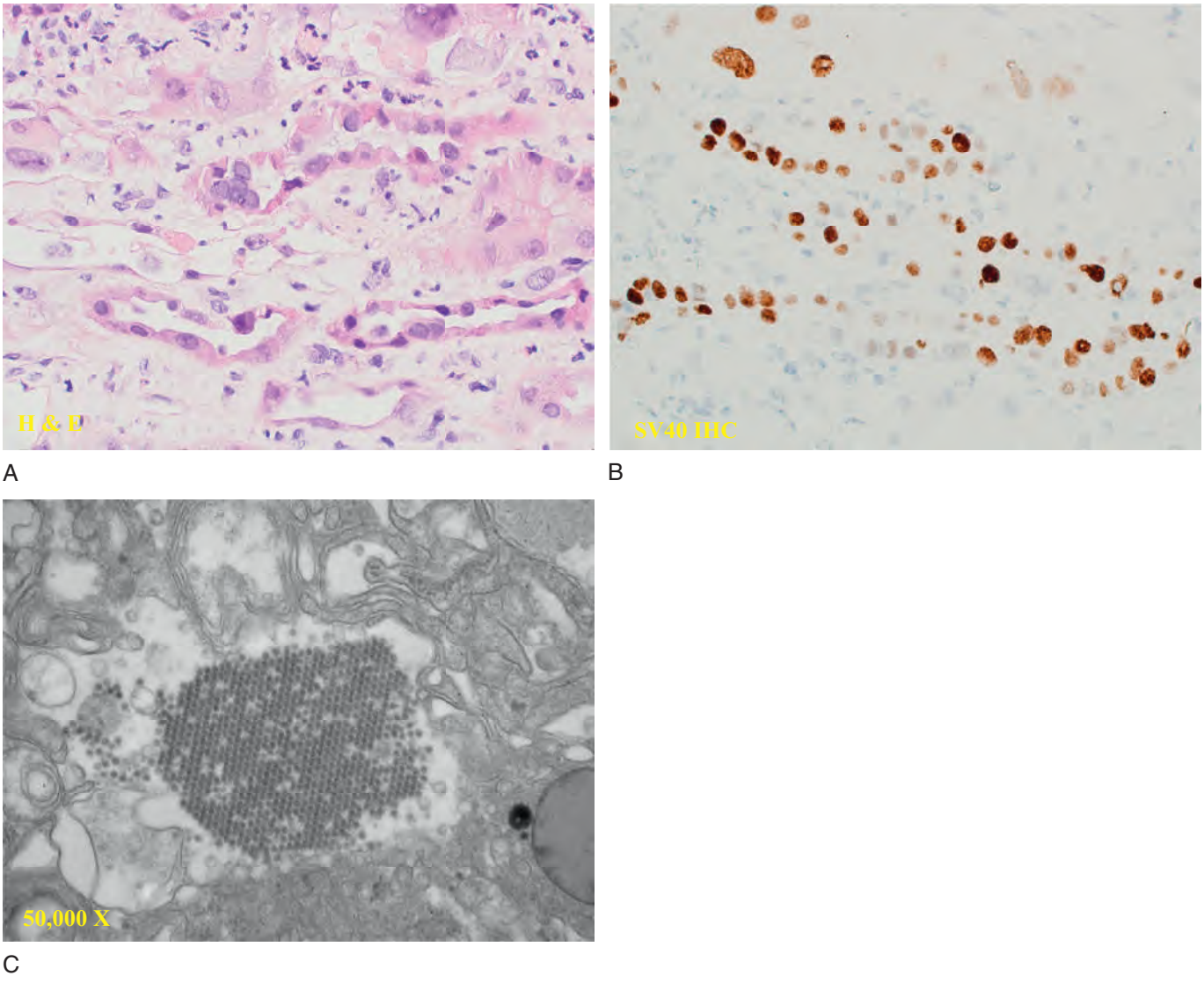

FIG. 144.4 Histology of BK virus nephropathy. (A) Hematoxylin and eosin stain demonstrating tubular epithelial cells with viral inclusions and interstitial inflammation. (B) Simian virus 40 (SV40) antibody stain showing BKV-infected cells. (C) Electron microscopy image showing BK virus. *(Courtesy I. Stillman, Department of Pathology, Beth Israel Deaconess Medical Center, Boston MA.)*

reducing BK viral load in the plasma of renal transplant recipients.[224–226] A randomized double-blinded study is currently underway to determine the efficacy of IVIG in treatment of BK viremia in renal transplant recipients. Lastly, potential future treatments include switching immunosuppression to mTOR inhibitors and changes in T-cell transfer of immunity.

Ureteral Stenosis

Post–renal transplant patients with the occurrence of BKV-associated ureteral stenosis can benefit from reduced immune suppression. Further treatments primarily involve surgical interventions that relieve the obstruction.

Hemorrhagic Cystitis

Treatment of hemorrhagic cystitis is symptomatic and includes continuous bladder irrigation, analgesia, hyperhydration, forced diuresis, and transfusion to maintain platelet levels above 50,000 cells/mm^3 and hematocrit values greater than 25%. Intravesicular cidofovir has been used in treatment of hemorrhagic cystitis in several case reports and retrospective studies.[227–232] Although this agent may provide some symptomatic relief, there is no definitive decrease of urine BKV load with treatment.[233] Randomized controlled trials are needed to clarify benefits of intravesicular cidofovir use in hemorrhagic cystitis. Prophylaxis with ciprofloxacin resulted in reduced incidence of BKV-associated hemorrhagic cystitis after HSCT in a small retrospective study.[234]

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145 Hepatitis B Virus

Chloe Lynne Thio and Claudia Hawkins

SHORT VIEW SUMMARY

Definition
- Hepatitis B virus (HBV) causes chronic hepatitis B, which can lead to progressive liver disease, including cirrhosis and hepatocellular carcinoma.

Epidemiology
- Nearly 5% percent of the world's population has chronic hepatitis B, but prevalence varies widely (see Table 145.1).
- The highest HBV prevalence is in Asia, Africa, and parts of the Middle East.
- Chronic hepatitis B is the leading cause of end-stage liver disease worldwide.
- Transmission of HBV is through percutaneous, sexual routes, or perinatal. Perinatal transmission is the leading cause of HBV worldwide.

Microbiology
- HBV is a partially double-stranded DNA virus in the Hepadnaviridae family that primarily infects hepatocytes.
- HBV replication is through an RNA intermediate, so it has a reverse transcriptase.

Clinical Presentation and Diagnosis
- Clinical presentation of HBV varies from asymptomatic to fulminant hepatitis with liver failure.
- The risk of developing chronic hepatitis B is inversely proportional to age of acquisition of infection. Chronic hepatitis B is also more likely in high-risk patient groups, such as injection drug users, men who have sex with men, and human immunodeficiency virus–infected individuals.
- Laboratory diagnosis of HBV is by enzyme immunoassays, which detect various HBV antigens and antibodies, and by real-time polymerase chain reaction to detect HBV DNA (see Table 145.2).

Therapy
- Anti-HBV agents significantly reduce complications of chronic hepatitis B, including liver cirrhosis and hepatocellular carcinoma. These therapies are also effective in reducing the recurrence of HBV infection in transplant recipients and other immunocompromised hosts.
- HBV is treated with 180 μg of pegylated interferon-α (PEG IFN-α) weekly for 48 weeks or with nucleos(t)ide analogues, often indefinitely (see Table 145.4).
- The first-line nucleos(t)ide agents are entecavir 0.5 mg daily or tenofovir disoproxil fumarate (TDF) 300 mg daily or tenofovir alafenamide 25 mg daily.
- New drugs are in early phase trials

Prevention
- Vaccination with the hepatitis B vaccine is recommended in all infants and children and for nonimmune adults at high risk for infection (see Table 145.6).
- Infants born to mothers with chronic hepatitis B should receive the hepatitis B vaccine and hepatitis B immune globulin (HBIG) at birth. Mothers with HBV DNA >1962 IU/mL should receive TDF in the third trimester of pregnancy to reduce the risk of transmission to the infant.
- Nonimmune individuals who have percutaneous, sexual, ocular, or mucous membrane exposure to HBV-infected fluids should receive postexposure prophylaxis with HBIG and hepatitis B vaccine.

OVERVIEW

Hepatitis B virus (HBV) infects more than 200 million people worldwide. It is the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC), and these sequelae of chronic infection account for more than 800,000 deaths annually. The outcome of infection and spectrum of illness varies widely. During the acute phase, infections range from asymptomatic hepatitis to icteric hepatitis, including fulminant hepatitis. Once chronic infection is established, the spectrum of illness ranges from the asymptomatic, inactive hepatitis to progressive liver disease that results in the sequelae of end-stage liver disease, including cirrhosis and HCC. Even though HBV cannot be cultured easily, enough is known about the viral life cycle that specific antivirals are available to control viral replication in patients; however, no cure is available yet for hepatitis B. Identification of the correlates of protective immunity has facilitated the widespread use of a highly effective vaccine, which by preventing chronic carriage of the virus and the subsequent development of HCC, represents the first true cancer vaccine.

Historical Background and Classification

Hippocrates recognized the spread of jaundice by infectious agents as early as 4000 BC. Recently, the oldest known HBV was isolated from the mummified remains of a child discovered in the 16th century from Naples, Italy.[1] The early modern cases of HBV infection were linked to the use of conventional viral vaccines, which were prepared from or contained human serum. In 1885 Lurman[2] described the appearance of jaundice in 15% of 1289 shipyard workers who received smallpox vaccine prepared from human lymph. Epidemics of hepatitis were also recorded after the administration of yellow fever vaccine, which was stabilized with human serum.[3] In the early part of the 20th century the increasing use of contaminated syringes and needles by diabetics on insulin and by patients treated for syphilis at venereal disease clinics elevated the importance of serum hepatitis.[4,5] This led to the association of hepatitis B with blood and blood products, as well as to its distinction from infectious hepatitis, caused by hepatitis A virus, a member of the Picornaviridae family.[6] The first hint of viral etiology came from the studies of Blumberg,[7] who reported the discovery of a human antigen in Australian Aboriginals termed Australian (Au) antigen. Subsequently, the Au antigen came to be known as hepatitis B surface antigen (HBsAg), and its association with acute hepatitis was established. For this discovery, Dr. Baruch Blumberg received the Nobel Prize in Physiology and Medicine in 1976. In 1971 Dane, an electron microscopist, visualized the presence of 22-nm HBsAg subviral particles along with the complete 42-nm virus particles in the blood of hepatitis B patients (Fig. 145.1).[8]

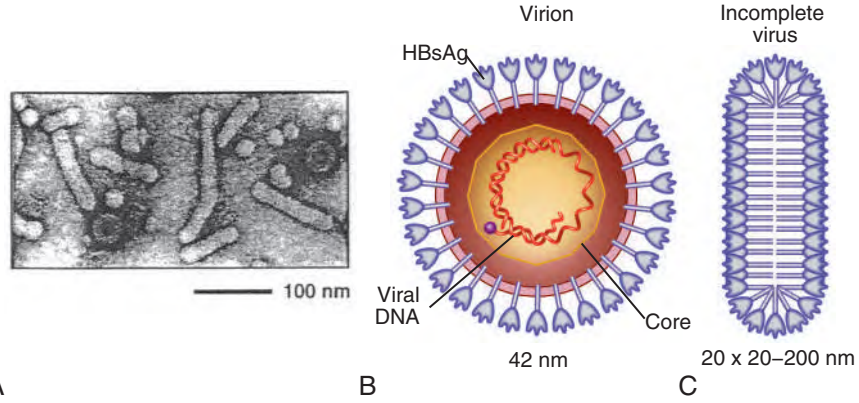

FIG. 145.1 Structure of hepatitis B virus (HBV) and hepatitis B surface antigen (HBsAg) particles. (A) Electron micrograph of negatively stained HBV. (B) Diagram of 42-nm HBV showing partially duplex DNA genome with a covalently linked protein at the 5′ end of the complete positive strand. (C) Diagram of 22-nm HBsAg filament. *(Modified from Flint SJ, Enquist LW, Racaniello VR, et al.* Principles of Virology. *2nd ed. Washington, DC: American Society for Microbiology Press; 2004:808.)*

Due to their unique biologic and molecular characteristics, as well as their liver tropism, HBV and HBV-like animal viruses were given the status of a new family designated Hepadnaviridae (*hepa*totropic *DNA vir*uses). HBV, the human pathogen, and other mammalian hepatitis viruses with sequence homology and similar genome organization are grouped in the genus *Orthohepadnavirus.* The genus *Avihepadnavirus* includes viruses that infect ducks, geese, and herons. The hepadnavirus animal models, which include duck hepatitis B virus,[9] woodchuck hepatitis virus, and ground squirrel hepatitis virus, have been extensively studied and have contributed to the current knowledge of the molecular biology of HBV infection and replication. However, these viral genomes are considerably divergent from human HBV, and there are limited tools to study the immune reaction to the virus in these animals. The primary host for HBV is human, but HBsAg has been detected in other primates, such as chimpanzees, gibbons, orangutans, African green monkeys, and squirrel monkeys. However, the use of these animals for the study of pathogenesis is both difficult and expensive. Thus a convenient animal model for HBV remains unavailable; however, the discovery of the HBV receptor on hepatocytes has led to development of a more efficient tissue culture system that should facilitate new drug discovery. HBV was molecularly cloned from patients' sera in 1979 and its complete DNA sequence determined.[10–12]

Virology

HBV is a small DNA virus, whose 3200-kilobase (kb) partially double-stranded DNA genome is maintained in a circular conformation.[13] Partially duplex DNA molecules represent incomplete synthesis of the viral DNA during morphogenesis (Fig. 145.2).[14] One of the unique features of HBV infection is the production of large quantities of subviral spherical and filamentous HBsAg particles, which are devoid of HBV DNA and so are noninfectious, in addition to complete virus particles. Under electron microscopy, these are distinguished by a diameter of 42 nm for complete virus (Dane) particles and 22-nm spherical and filamentous structures for subviral particles (see Fig. 145.1).[8] HBsAg is expressed on the exterior of all these particles. HBsAg subviral particles reach a titer of 10^{13}/mL, whereas viral particles range in titer from 10^{4}/mL to 10^{9}/mL. All of these particles circulate in blood and permit convenient diagnosis of viral antigen by enzyme-linked immunosorbent assay or radioimmunoassay.[15] HBsAg forms the viral component of the lipoprotein envelope, which encloses a core shell containing the viral DNA genome and the virus-encoded polymerase protein. The nucleocapsid or core is composed of a 21-kilodalton (kDa) basic phosphoprotein commonly known as hepatitis B core antigen (HBcAg).[16] A cell-derived kinase activity has been shown to be associated with the virion particles,[17] but the functional significance of this enzyme is not understood.

The subviral particles are composed of surface antigen polypeptides and lipids derived from hepatocyte membranes. The lipid content is approximately 30% by weight and includes phospholipids, cholesterol, cholesterol esters, and triglycerides.[18,19] Because of their high immunogenicity, purified HBsAg particles can be used as an HBV vaccine. HBsAg elicits neutralizing antibodies, which offer protection from reinfection.[20] The high titers of HBsAg in patients during natural infection can potentially serve to adsorb neutralizing antibody or promote T-cell exhaustion, thus protecting the virus from host defenses.[21]

Attachment, Entry, and Hepatotropism

HBV primarily infects hepatocytes. The pre-S1 domain of the large HBsAg protein promotes attachment and entry of HBV into the hepatocyte via a liver cell–specific receptor recently identified as Na (sodium) taurocholate cotransporting polypeptide (NTCP), which is an integral membrane protein used in bile acid transport.[22,23] There is evidence that hepatocyte entry may be a multistep process, including binding to heparan sulfate proteoglycans,[24] which are found on a variety of cells, and clathrin-mediated endocytosis.[25] The viral envelope fuses with the endosomal membrane and delivers core particles into the cytoplasm, which are transported to the nuclear pore complex, allowing viral DNA to enter the nucleus.[26] In the nucleus the partial strand is repaired, forming a double-stranded covalently closed circular (ccc) DNA form (Fig. 145.3), which is the template for replication (see "Replication").[9] Liver specificity is also displayed at the level of viral gene expression, which is controlled largely by the promoters and enhancers (see Fig. 145.2).

Viral Genome

HBV uses unique transcriptional and translational strategies to maximize the limited coding capacity of its genome. The HBV DNA consists of the complete (negative) strand, which contains a protein covalently linked to its 5′ terminus, and the incomplete (positive) strand, displaying variable length and bearing a 5′ capped oligoribonucleotide (see Figs. 145.1 and 145.2).[27,28] The asymmetry of the DNA strands reflects the incomplete synthesis of DNA during maturation of viral particles. HBV DNA codes for four overlapping open reading frames (ORFs): S, for the surface antigen or envelope gene (HBsAg); C, for the nucleocapsid (core), and "e" antigen gene (HBcAg, HBeAg); P, for the polymerase gene; and X, for the HBx gene (see Fig. 145.2). The surface antigen ORF contains three in-frame initiator codons from which small or major (S), middle (M), and large (L) kDa HBsAg polypeptides are synthesized, respectively. These polypeptides are variably glycosylated to yield several species: S, p24/gp26; M, p30/gp33/gp37; and L, p39/gp42 kDa HBsAg polypeptides, respectively. L and M HBsAg contain characteristic pre-S1 and pre-S2 domains, and hence these proteins are also referred to as pre-S1 and pre-S2 proteins, respectively. The subviral particles are composed of mostly S and lesser amounts of M polypeptides and few or no L polypeptides. The Dane particles, which represent the complete virus, contain all three HBsAg forms.

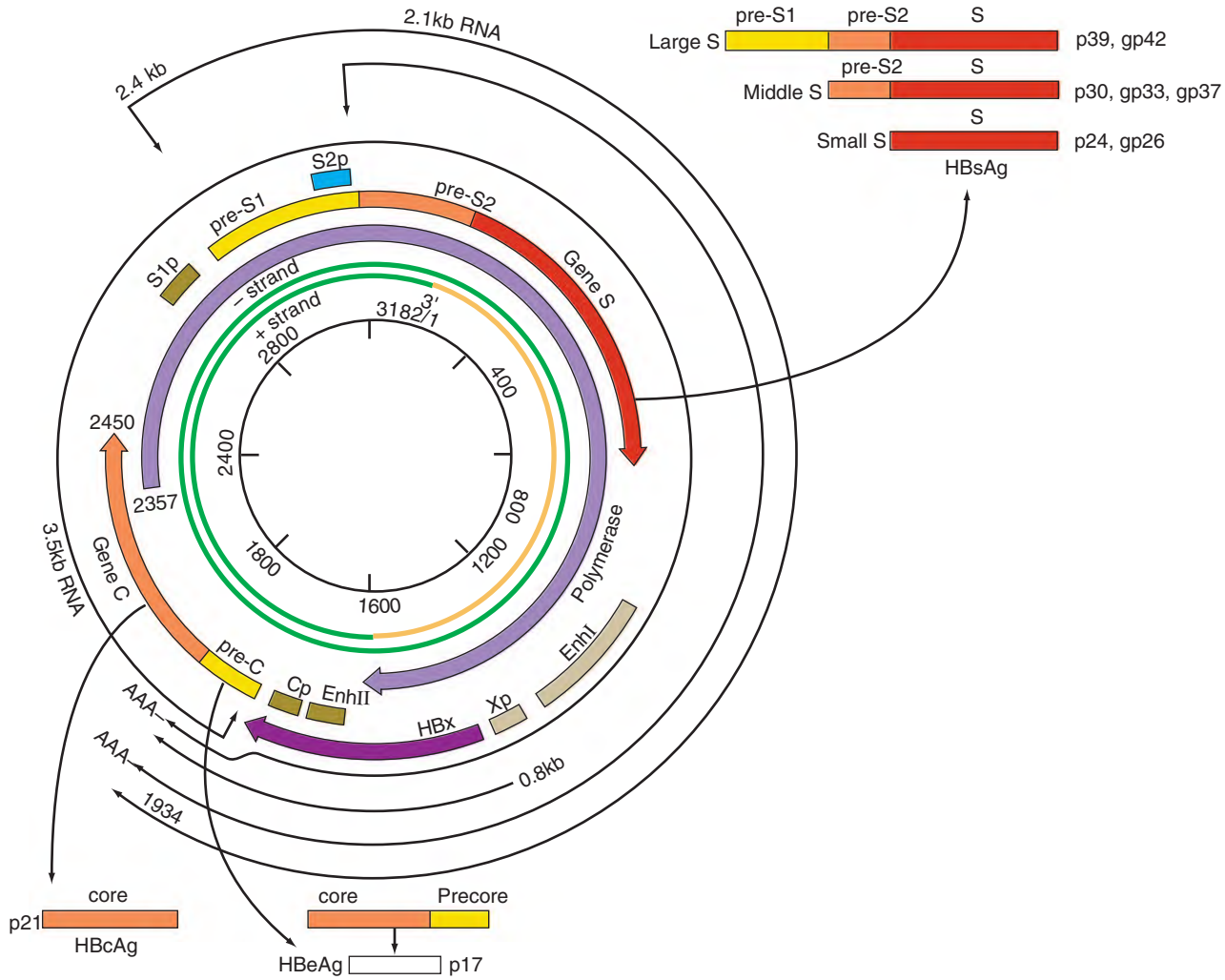

FIG. 145.2 Hepatitis B virus (HBV) genome organization, map of viral transcripts, and proteins. The partially double-stranded 3.2-kilobase viral DNA is shown in the inner circle. The single-stranded (ss) region is indicated in yellow-orange. The extent of the ss region varies from molecule to molecule. HBV-encoded overlapping genes are indicated in the outer circles in various colors. Four promoter regions preceding a corresponding gene are indicated as S1p, S2p, Cp, and Xp. Two enhancer elements (I and II) are also shown. Viral transcripts are indicated in the outermost circles *(thin lines).* The three forms of HBsAg, HBcAg, and HBeAg (surface, core, and e antigen, respectively) polypeptides are also shown. *(Modified from Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Hepatitis viruses. In: Murray PA, Rosenthal KS, Kobayashi GS, et al, eds.* Medical Microbiology. *4th ed. St. Louis: Mosby; 2002:591–605.)*

The C region, or the core ORF, contains two in-frame initiator codons, with the first adenine-thymidine-guanine (ATG) start codon responsible for e antigen polypeptide synthesis (HBeAg) (see Fig. 145.2). The second ATG serves as an initiation codon for the HBcAg polypeptide. HBeAg is secreted from cells and accumulates in serum as an immunologically distinct soluble antigen and serves as a marker of ongoing viral replication. Both gene products (core and e polypeptides) are made from the same reading frame. The P and X ORFs encode the polymerase and the HBx proteins, respectively.

Before the advent of current molecular methods, HBV was divided into serotypes on the basis of the antibody response to HBsAg. However, HBV is now divided into 10 genotypes (A–J), based on genetic diversity of at least 8% in the HBV genome.[29,30] Within these genotypes are subgenotypes, which differ by a minimum of 4%. These genotypes and subgenotypes are distributed geographically, with genotypes A in North America, Europe, and parts of Africa; genotypes B and C in Asia; genotype D in India, the Middle East, the Mediterranean region, and parts of Africa; genotype E in Africa; genotype F in Central and South America; genotype G in France, Germany, and North America. Genotype H is found in Central America. Genotype I, a novel recombination between genotypes A, C, and G, is found in Vietnam and Laos.[31] Genotype J was identified in Japan.[32] There are accumulating data that these genotypic differences are associated with disease and treatment outcomes. Genotypes G and C are associated with more severe liver disease and higher HCC risk.[33] Genotype C also has slower HBeAg seroconversion compared with genotype B.[34,35] Genotypes A and B are more responsive to pegylated interferon-α (PEG IFN-α) therapy than are genotypes C and D.[36]

Transcription

The HBV genome maximizes the use of its limited genomic size by using multiple overlapping reading frames and multiple initiation codons to generate antigenically different proteins. The cccDNA is a stable episomal form of HBV in the nucleus that is associated with histones and other proteins, serves as the substrate for viral transcription, and uses host polymerase II (pol II) in the synthesis of viral transcripts (see Fig. 145.3). The expression of the four different genes, designated C, S, P, and X, is regulated by four promoter elements (S1p, S2p, Cp, and Xp) and two enhancer elements (enhancer I and II; see Fig. 145.2).[15] These transcriptional regulatory elements direct the synthesis of multiple viral transcripts that are approximately 3.5, 2.4/2.1, and 0.8 kb in length, respectively. All viral transcripts

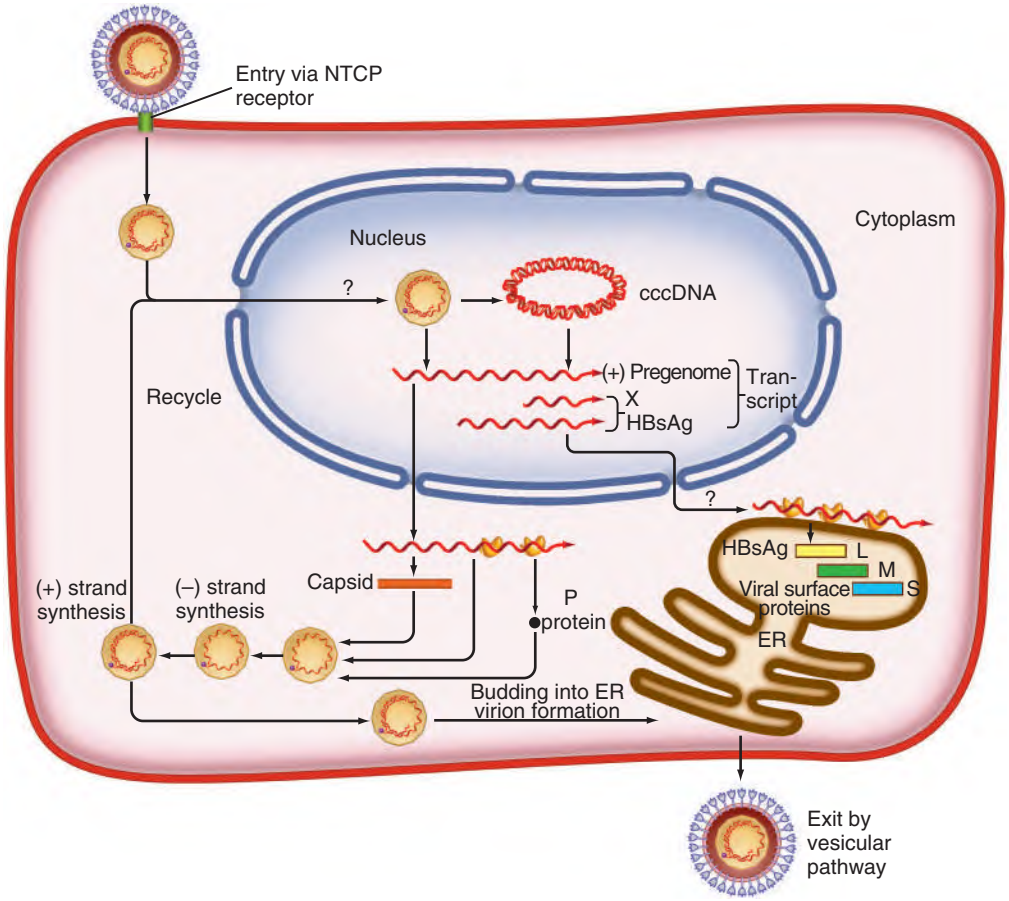

FIG. 145.3 Hepatitis B virus life cycle. The virion attaches to the hepatocyte through the Na (sodium) taurocholate cotransporting polypeptide *(NTCP)* receptor. It is brought into the cytoplasm via clathrin-mediated endocytosis. The surface protein binds to the endosomal membrane, allowing release of the capsid, which is transported to the nuclear pore complex, where the capsid dissociates and the DNA is translocated to the nucleus. In the nucleus the partial double strand is completed, forming a covalently closed circular form called *cccDNA*. The negative strand of such cccDNA is the template for transcription by cellular RNA polymerase II of a longer-than-genome-length RNA, called the *pregenome,* and shorter subgenomic transcripts. Viral messenger RNAs (mRNAs) are transported from the nucleus. The hepatitis B surface antigens *(HBsAgs)* encoding viral mRNAs are translated by ribosomes bound to the endoplasmic reticulum *(ER),* and the proteins enter the secretory pathway. The pregenome RNA is translated at low efficiency to produce a 90-kilodalton polymerase protein, *P,* which possesses reverse transcriptase activity. This protein then binds to a specific site at the 5′ end of its own transcript, where viral DNA synthesis is eventually initiated. The pregenomic RNA also serves as mRNA for the capsid protein. Concurrently with capsid formation, the RNA-P protein complex is packaged and reverse transcription begins with synthesis of negative-strand DNA, followed by positive-strand DNA synthesis. Before the completion of +/– strand synthesis, core particles mature, and these structures acquire envelopes by budding into the ER, where viral morphogenesis is completed. Progeny-enveloped virions are released from the cell by exocytosis or are recycled to the nucleus, where the process is repeated. *(Modified from Flint SJ, Enquist LW, Racaniello VR, et al.* Principles of Virology. *2nd ed. Washington, DC: American Society for Microbiology Press; 2004:809.)*

are unspliced, capped, and polyadenylated. All the transcripts are encoded on one strand of the DNA and coterminate at an identical polyadenylation site.[37]

The pre-S1 (S1p) promoter directs the synthesis of the 2.4-kb transcripts, which code for the large envelope (L HBsAg) polypeptide. This promoter contains binding sites for two liver-enriched transcription factors, hepatocyte nuclear factor 3 (HNF-3) and HNF-1, which are primarily responsible for the liver-specific activity of this promoter.[38] The pre-S2 or S (S2p) promoter directs the transcription of multiple species of messenger RNAs (mRNAs) coding for pre-S2/M and S HBsAg polypeptides. Collectively, these RNA species are approximately 2.1 kb in length with 5′ heterogeneous ends. The mRNAs, which initiate downstream of the pre-S2 initiator ATG, code for the S, or the major polypeptide. The S2p promoter also displays liver specificity and is controlled by the enhancer II element located about 2000 base pairs away (see Fig. 145.2).[39] The S2p promoter is stronger than the S1p promoter, which results in the synthesis of an excess of the major S over the L (pre-S1) and M (pre-S2) forms of the HBsAg. This regulation is especially critical for synthesizing the appropriate levels of the three forms of surface proteins within the cell. The basis for the differential regulation of these promoters is governed by mechanisms that involve both positive and negative *cis*-acting elements and the *trans*-acting transcriptional factors.[15,40]

The core/pregenomic promoter (Cp) governs the expression of two longer-than-genome-length transcripts (3.5 kb) designated precore (pre-C) and core (C) RNAs. The slightly longer precore RNA directs the translation of HBeAg polypeptide. The shorter C RNA is used for the translation of core and polymerase proteins. The ATG for the polymerase (reverse transcriptase) is located several hundred nucleotides from the 5′ end (see Fig. 145.2). The C RNA, after its translation into core polypeptides, packages its own RNA, which then functions as a pregenome RNA (pregenomic RNA). The Cp promoter contains binding sites for several liver-enriched and ubiquitous transcription factors.[40] Immediately juxtaposed to this lies enhancer II, which, in concert with enhancer I, plays a regulatory role in the overall HBV gene expression. A complex scheme of transcriptional regulation appears to operate within these control elements.[41]

The X promoter (Xp) is located immediately downstream of enhancer I and regulates the synthesis of the low abundance 0.8-kb X transcripts that correlate with similar levels of HBx protein synthesis in infected

cells.[42,43] Both ubiquitous and liver-enriched factors binding to enhancer I regulate the biosynthesis of X transcripts.

Translation

HBV, which encodes three major viral transcripts, also uses unique translational strategies to maximize the limited coding capacity of its genome. The S region contains three in-frame translational start codons from which the synthesis of three distinct surface antigen polypeptides is regulated (L/pre-S1, M/pre-S2, and S). These proteins exhibit distinct amino termini and differ with respect to the extent that they are post-translationally modified by glycosylation.[15] The S protein is referred to as the major surface antigen because it represents approximately 85% of the HBsAg that is produced by the virus. The pre-S1 (L) and pre-S2 (M) proteins are present at an abundance of approximately 15% and 1% to 2%, respectively.

The pre-C and C mRNAs are translated into e and core polypeptides (HBe/cAg), respectively. The pre-C protein contains a signal peptide sequence encoding 19 amino-acid residues, which targets this protein to the secretory pathway in the endoplasmic reticulum (ER).[44] Further processing of the protein includes cleavage of the signal peptide, and several amino-acid residues at the carboxyl terminus, leading to the production of a 17-kDa HBeAg (see Fig. 145.2). HBeAg is secreted and found in the serum of patients and serves as a marker of active replication in chronic hepatitis. Although the function of HBeAg is not clearly understood, one study demonstrated that it downregulated Toll-like receptor 2 expression on hepatocytes and monocytes, leading to a decrease in cytokine expression.[45] These data suggest a role for HBeAg in modifying the innate immune response to HBV. HBeAg is dispensable for replication, as HBV carriers with pre-C mutant viruses arise spontaneously and are both infectious and pathogenic.[46,47] The C RNA initiates downstream of the pre-C ATG and translates into HBcAg and less frequently into polymerase. Because polymerase ATG is located several hundred nucleotides downstream of the 5′ end (see Fig. 145.2), the ribosomes must access this initiator codon by a mechanism different from ribosome scanning. Because this process is inefficient, one polymerase protein is translated for every 200 to 300 molecules of core polypeptides.

HBV polymerase protein consists of 832 amino acids and is composed of four domains: terminal protein (TP), spacer, reverse transcriptase (RT), and ribonuclease H (RNase H).[15] The TP domain represents the portion of the polymerase protein that is covalently bound to negative-strand DNA. The spacer domain or tether region connects the TP domain to the RT, which contains the catalytic domain for the polymerase. The RT domain is followed by an RNase H domain, which functions to hydrolyze the pregenomic RNA template after reverse transcription within the nucleocapsid.[48]

Replication

Although HBV is a DNA-containing virus, the replication occurs via an RNA intermediate, a characteristic that places hepadnaviruses close to retroviruses. Much of our understanding of HBV replication strategy comes from the classic experiments of Summers and Mason,[49] which demonstrated that HBV amplifies via reverse transcription of an RNA intermediate and that these events occur within the subviral core particles in the cytoplasm. The complex mechanism by which pregenomic RNA is converted to partially double-stranded virion DNA begins with the encapsidation of pregenomic RNA by the core polypeptide along with the polymerase (Fig. 145.4).[50,51] The pregenomic RNA is greater than genome length because it contains terminally redundant 200-nucleotide sequences, which include an epsilon (ε) stem-loop structure, and a short sequence of 11 to 12 nucleotides termed DR1 (see Fig. 145.4).[15] However, only the 5′ end ε stem-loop structure contains a binding site for the HBV polymerase to bind to its own molecule. The interaction between the ε signal of the pregenomic RNA and the polymerase leads to the formation of a three-base DNA priming sequence. This primer sequence then transfers to the DR1 near the 3′ end of the pregenomic RNA (pgRNA) to initiate elongation of the negative-strand DNA and concurrent digestion of pgRNA by RNase H of the polymerase. After this, positive-strand synthesis occurs. The positive strand is extended to variable lengths to yield mature HBV DNA. The cessation of the positive-strand synthesis at various stages of its synthesis coincides with the maturation of the core particles and their entry into the ER, leading to the packaging of partially duplex genomic DNA found in hepatitis B (HB) virions (see Fig. 145.3). This entry prevents access to cytoplasmic deoxynucleotide pools needed for completion of DNA synthesis. Matured core particles face two options at this stage: They either enter the ER, undergo virion packaging with HBsAg polypeptides, and bud from the membrane as 42-nm Dane particles; or they enter the nucleus and deliver the partially duplex DNA and repeat the cycle of replication (see Fig. 145.3). cccDNA serves as a substrate of all viral mRNA synthesis; it is the stable form of HBV DNA that is most resistant to antiviral treatment and the host immune response.[15] In summary, the salient features of HBV replication are cccDNA as the replication template for all viral RNAs, the use of pregenomic RNA as a template for reverse transcription occurring within core particles; incomplete positive-strand synthesis that results in a partially duplex DNA genome found within the hepatitis B virion particles; and mature core particles either enter nucleus or are secreted out of the hepatocyte.[52]

HBx

HBx is a regulatory protein whose primary role is to enhance transcription of the HBV genome by promoting degradation of a host restriction factor, Smc5/6.[53] It is also involved in several cellular pathways. The cellular targets are numerous, and the list of the cellular functions HBx has been shown to modulate continues to grow.[15] Overall, the functions of HBx are characterized as transcriptional *trans*-activators. Nuclear factor kappa B (NF-κB) was one of the first HBx-responsive elements identified.[54] Because the NF-κB site does not exist within the HBV genome, it is believed that the *trans*-activating potential of HBx extends to cellular target genes involved in inflammation. Other HBx-responsive transcription factors include *a*ctivating *t*ranscription *f*actor 2/*c*AMP *r*esponse *e*lement *b*inding protein (ATF-2/CREB), activation protein 1 (AP-1), signal transducer and activator of transcription (STAT)-3, and nuclear factor of activated T cells (NF-AT).[55,56] Within the cytoplasm, it also localizes to the mitochondria and induces oxidative stress via calcium signaling.[57,58] These activities may mediate mitochondria-mediated liver injury. HBx has also been shown to modulate cellular signal transduction pathways.[59,60] It was shown to activate sarcoma (Src) kinase and stimulate the Ras-Raf–mitogen-activated protein kinase (MAPK) signal transduction pathway.[61] The physiologic significance of any of these observations remains to be established in the context of human infections. HBx is not known to be directly oncogenic, but on the basis of its role in activation of transcription factors, elevation of reactive oxygen species, and inhibition of apoptosis,[62] it is considered a potential contributor to the processes of liver oncogenesis.[63]

Morphogenesis and Assembly

Envelopment of the nucleocapsid occurs in the ER or ER-Golgi intermediate compartments.[15] All three forms of the surface proteins are synthesized in the ER as integral transmembrane proteins. M protein is not important for morphogenesis but is required for infectivity. The N-terminal domain of L is critical for virion morphogenesis, serving to translocate the newly-synthesized viral cores across the ER membranes[64] After budding from the ER the virions are secreted via vesicular transport through the remaining compartments of the secretory pathways and are eventually released into the bloodstream. During chronic hepatitis, accumulation of the L surface protein–containing particles has been associated with the pathologic phenotype of ground-glass hepatocytes.[15]

NATURAL HISTORY AND PATHOGENESIS OF DISEASE

Natural History

If HBV is acquired in adulthood, ~95% mount a successful immune response that results in recovery and production of protective antibodies (anti-HBs). In contrast, this only occurs in 5% to 10% of perinatally acquired infection. However, recovery is not synonymous with viral clearance because cccDNA is still present in some hepatocytes, so reactivation can occur (see "Reactivation").

The phases of chronic hepatitis B are in flux, with the newest guidelines delineating phases based on serologic markers. These phases result from the interplay between the virus and the host; however, it is not necessary

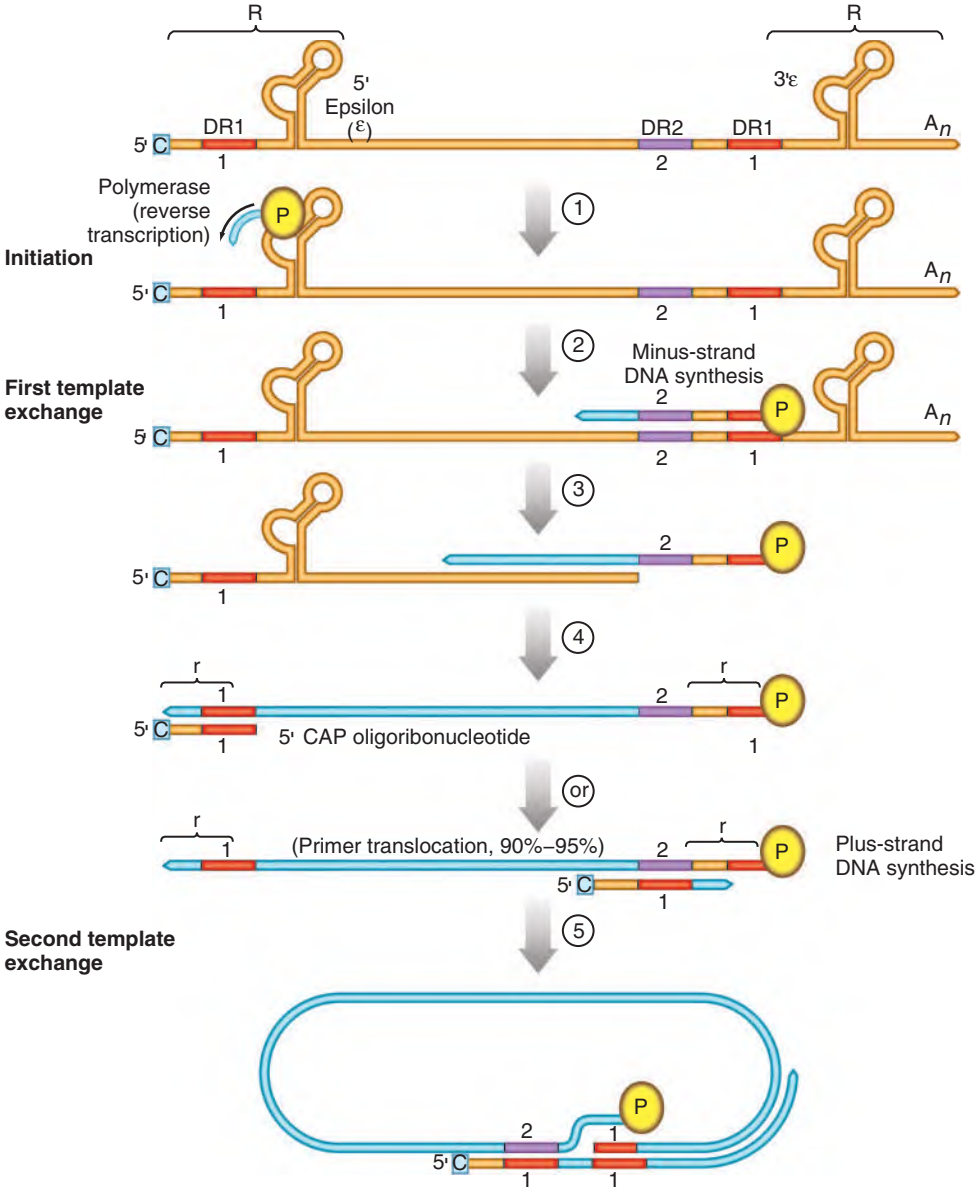

FIG. 145.4 Hepatitis B virus replication pathway. The terminally redundant pregenomic RNA (pgRNA) *(top line)* is capped and polyadenylated. Boxes 1 and 2 represent *DR1* and *DR2*, respectively. The horizontal bracket labeled *R* represents the terminally redundant region of the RNA. The epsilon (ε) stem-loops are indicated at the termini. pgRNA packaging into cores is initiated by the interaction of polymerase *(P)* protein with the 5′ copy of ε, which leads to formation of 3–base pair priming sequence. This primer sequence translocates to the DR1 of the 3′ end of the pgRNA, initiating positive synthesis (step 2). As DNA synthesis continues the pgRNA template is degraded by the associated ribonuclease H activity of the polymerase (step 3). Elongation of negative-strand DNA is finished on complete copying of the pgRNA template (step 4). The product is a terminally redundant complete negative-strand DNA species. The redundancies (8–10 nucleotides) are labeled *r*. At this time the primer for positive-strand synthesis is generated from the 5′-terminal 15 to 18 nucleotides of the pregenomic RNA. The primer is capped *(CAP)* and includes the sequence to the 3′ end of DR1. Elongation of the positive strand results in a duplex linear genome. In the majority of cases the primer is translocated and base pairs with the DR2 sequence near the 5′ end of negative-strand DNA. The positive-strand synthesis is initiated, and then elongation begins. On reaching the 5′ end of negative-strand DNA, an intramolecular template exchange occurs, resulting in a circular DNA genome (step 5). This exchange is facilitated by the short terminal redundancy in negative-strand DNA. The positive-strand DNA synthesis then continues for a variable distance, resulting in the relaxed circular partially duplex form of the genome found in mature virions. *(Modified from Tavis JE. The replication strategy of the hepadnaviruses.* Viral Hepatitis Rev. *1996;2:205–218.)*

to go through all of the phases.[65] The first phase, the HBeAg-positive HBV infection phase (also known as the immunotolerant phase), is characterized by limited immune response against the virus, so there is minimal elevation of serum aminotransferases and minimal liver inflammation despite circulating HBsAg, HBeAg, and high levels of HBV DNA. In cases of perinatal infection, this phase may last for decades, during which there are low rates of spontaneous seroconversion to anti-HBe. The cumulative rate of spontaneous HBeAg clearance during this phase is estimated to be approximately 2% during the first 3 years of life and only 15% 20 years after perinatal infection.[66,67] In contrast, among immunocompetent adults, this phase is typically present only during the incubation period.

During the second phase of the infection the HBeAg-positive chronic hepatitis B phase (also known as the immune active phase), there is an increase in immune response, accompanied by increased aminotransferases and liver inflammation and high HBV DNA levels. This is thought to be the period in which there is augmentation of both innate and acquired HBV immunity, leading to cytolytic destruction of hepatocytes, as the peak of the immune response coincides with the aminotransferase elevations.[68] The result of this increased immune response is usually

conversion from HBe antigenemia to anti-HBe. In perinatally acquired infection, this period usually occurs in the second to third decade of life, and HBeAg seroconversion rates may approach 10% to 20% per year.[66,69] Individuals with active chronic hepatitis B remain in this phase because the immune reaction is not sufficiently vigorous to result in production of protective antibodies and viral control. Some individuals with chronic hepatitis B can eventually experience HBeAg seroconversion. In a study of 1536 Alaskan natives who acquired HBV as adults and did not receive treatment, two-thirds of subjects cleared HBeAg and developed anti-HBe during 12 years of follow-up without reversion to the HBeAg-positive state.[70] The likelihood of seroconversion is inversely related to the serum aminotransferase. Spontaneous seroconversion occurs in 50% of those with serum aminotransferases greater than five times the upper limit of normal (ULN), compared with <10% in those with lower aminotransferases.[71] Spontaneous seroconversion is marked by the appearance of HBcAg-specific $CD4^+$ and $CD8^+$ T cells,[72] but the precise factors preceding this event are unknown, and seroconversion cannot be linked to specific viral mutants, as was previously believed.[73] Males and older individuals are also more likely to have spontaneous seroconversion, and viral genotype may play a role.[35] Because immunoglobulin M (IgM) anti-HBc may increase with alanine aminotransferase (ALT) flares during this phase, acute hepatitis B may be erroneously diagnosed.

The third phase, known as the HBeAg-negative HBV infection phase (also known as the inactive phase), is characterized by absence of HBeAg, presence of anti-HBe, normal aminotransferases, and low or undetectable HBV DNA. Termination of virus replication is associated in most patients with biochemical and histologic regression of inflammatory activity.[74] Some individuals will also clear HBsAg, although this is unusual, occurring in <1% of patients per year in adults and 0.05% to 0.8% in infection acquired in infancy or childhood.[70] Individuals who remain in this phase have excellent prognosis and minimal liver injury. During this phase, seroreversion to HBeAg positivity and return to the second phase occurs 10% to 40% of the time.

The fourth phase is the HBeAg-negative chronic hepatitis B phase, where HBeAg remains negative but viral replication increases, resulting in high levels of HBV DNA and elevations of ALT. During this phase the HBV DNA levels are moderately high but generally lower than in those with HBeAg-positive chronic hepatitis B.

Pathogenesis

It has been difficult to understand the mechanism for viral persistence and immune-mediated liver injury because research in this area is hampered by the lack of a robust culture system or a small animal model. Most studies suggest that HBV is not directly cytopathic to the hepatocyte,[76,77] which is also supported by the existence of asymptomatic hepatitis B carriers with normal liver histology and function. Both human and animal studies have now shown that the liver injury mediated by HBV is initiated by a virus-specific cellular and humoral immune response. In >95% of immunocompetent adults the immune response is vigorous, polyclonal, and multispecific and results in acute, self-limited hepatitis with reduction of viral load and the development of long-lasting humoral and cellular immunity. Persistent infection is associated with necroinflammatory activity, which eventually leads to cirrhosis.

Acute Hepatitis B

Natural recovery from acute hepatitis B probably depends on multiple components of cellular immune responses, including natural killer (NK) cells, natural killer T (NK-T) cells, and virus-specific $CD4^+$ T cells and $CD8^+$ cytotoxic T lymphocytes (CTL). However, a hallmark of acute hepatitis B is failure to induce an innate cytokine response mediated by pattern recognition receptors.[78,79] In self-limited infections the noncytopathic response leads to lowering of the HBV DNA by >90% within a few weeks of peak HBV replication and before liver damage occurs.[80] Both NK and NK-T cells also contribute to clearance through production of IFN-α/β.[81] Acute HBV infection is also accompanied by a strong and transient expansion of $CD4^+$ T cells directed against multiple epitopes within the HBV. HBc is the dominant antigen recognized by $CD4^+$ T cells in most cases of acute, resolving HBV infection.[82] These HBc-specific $CD4^+$ T cells provide help for the production of antibody to HBsAg and are also associated with the development of a vigorous and polyclonal cytotoxic CTL capable of recognizing different epitopes within the HBV genome.[83] Individuals who successfully resolve an HBV infection, either spontaneously or after IFN therapy, maintain these broad and strong peripheral CTL responses.[84] $CD4^+$ and CTL memory in the presence of low levels of persisting HBV DNA has been shown to persist up to 23 years after infection despite markers of serologic recovery. Both virus-specific $CD4^+$ and CTL contribute to control of HBV replication through both direct cytolysis of infected cells but, more important, through production of cytokines that control viral replication.[85] Analysis of the NK, CTL, and $CD4^+$ T-cell responses in the incubation phase has demonstrated that CTL response increases in parallel with ALT, consistent with the previously observed notions that CTL activity is responsible for liver injury, and peaks about the time that the HBV DNA titers begin to fall.[86] Recently, regulatory T cells were found to influence the immune response during acute HBV infection by decreasing cytokine production of effector T cells, thereby down-regulating the antiviral activity. This limits the liver injury during acute infection but, as a consequence, prolongs viral clearance.[87]

The development of surface antibody (anti-HBs) follows the disappearance of HBsAg and marks recovery from acute hepatitis B, which occurs in 95% of adults after acute infection. Anti-HBs is sufficient for protection against HBV infection, as demonstrated by the success of the current HBV vaccines, even if it is not the sole operative mechanism clearing acute infection. The "a" determinant is the predominant B-cell epitope common to all HBV subtypes. Antibodies against this epitope confer immunity to all HBV subtypes. Coexistence of HBsAg and anti-HBs is reported in up to 24% of chronically infected individuals, in which case the anti-HBs is directed against one of the subtypic determinants, and a mutation preventing neutralization of the virus has occurred. Other surface antigens that stimulate antibody responses include the pre-S1 and pre-S2 antigens. Antibody to these develops during recovery and can be detected before anti-HBs; however, routine serologic assays are not readily available. Mutation in the S gene usually occurs in the "a" determinant, which allows the virus to escape antibody neutralization. This may occur after the use of hepatitis B immune globulin (HBIG) for passive immunization of newborns and transplant recipients.

Chronic Hepatitis B

Persistent HBV infection may result because of the failure of initial innate and adaptive immune responses. In animal models HBeAg may be tolerogenic,[88] and because HBeAg and HBcAg are cross-reactive at the T-cell level, deletion of the $CD4^+$ HBc-specific T-cell responses results in ineffective CTL responses to HBcAg.

Adult immunocompetent individuals who fail to resolve acute HBV infection also have less vigorous $CD4^+$ T-cell and CTL responses. In contrast to acute resolving infection, peripheral $CD4^+$ T-cell and CTL responses in those individuals who develop chronic infection are weak and more narrowly focused.[89] Despite these weak CTL responses, HBV-specific $CD4^+$ and $CD8^+$ T cells can be isolated from the livers of chronic hepatitis B patients that are capable of ex vivo, class-I–restricted cytolytic activity in response to envelope and core peptides. The cytotoxicity mediated by these cells appears to be sufficiently strong to cause liver injury but not strong enough to eradicate virus and cccDNA from all hepatocytes.[90,91] In addition to the virus-specific cells, the inflamed liver contains other cells that may participate in hepatocyte damage.[92] $CD8^+$ T cells from patients with chronic hepatitis B exhibit multiple coinhibitory molecules, including programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4). Upregulation of these exhaustion markers may occur in the liver microenvironment, where high HBV antigen concentrations exist, because one study found that HBV-specific $CD8^+$ T cells in the liver express higher levels of PD-1 compared with circulating T cells.[93]

The mechanisms by which HBV evades the immune response and results in chronic infection in these adults remain obscure. In part this is determined by host genetic factors because persons with certain genetic polymorphisms and human leukocyte antigen (HLA) alleles appear to be more susceptible to chronic infection,[94–97] and certain HLA-DP alleles have been associated with recovery from acute HBV infection.[98] It has

also been suggested that the size of initial viral inoculum and viral kinetics may be such that the immune system is overwhelmed by the virus and becomes "exhausted."[99]

Hepatocellular Carcinoma

Although epidemiologic evidence supports the role of HBV as a causal agent of liver cancer (see "Epidemiology of Hepatitis B" later),[100] the molecular mechanisms addressing the link between the viral infection and the development of HCC have been highly debated.[101] Despite a large body of work on this subject, a clear view of how HBV infection triggers events that lead to liver oncogenesis remains elusive. HCC development, like other cancers, proceeds in multiple steps that correlate with specific lesions associated with livers of patients with HCC. These include altered hepatic foci, dysplastic (neoplastic) nodules, and low- and high-grade HCCs. These lesions are characterized as exhibiting different levels of cell differentiation. Similar to other well-characterized human cancers, HCC progresses through these individual stages. The molecular switches associated with each step of HCC development still need to be identified.

HBV-related HCCs are derived from the clonal expansion of a single transformed cell or cancerous cells.[100] Whereas 80% of HBV-associated HCC tumors contain integrated viral DNA, most of the HBV genes are either truncated or transcriptionally inactivated.[15] After integration into the host chromosomes, the HBV DNA control elements, such as enhancers or promoters, can act in *cis* to activate a cellular oncogene. HBV integration was initially thought to be a random event, but recent next-generation sequencing has identified recurrent sites for integration alteration of the *TERT, MLL4, CCNE1, NTRK2, IRAK2,* and *p42MAPK1* genes, all of which are important for cell growth and differentiation.[102] It is thought that the random but multiple integration events might frequently lead to genomic instability by causing chromosomal aberrations, such as amplifications, translocations, and deletions, all of which have the potential for activating pathways that lead to liver neoplasia. Indeed, in HBV-associated liver tumors, the HBV DNA integrants are highly rearranged with deletions, inversions, and sequence reiterations.[103] HBV DNA insertion into cellular genes encoding for proteins that are necessary for control of cell signaling and proliferation has been demonstrated.[104]

The pleiotropic functions of HBx in HCC have been implicated in the processes of liver oncogenesis.[105] HBx modulates the expression and activities of numerous genes, as well as epigenetic molecules (e.g., microRNAs) and events (e.g., methylation and acetylation), leading to deregulation of various pathways and functions. Other functions include its effects on DNA repair processes, antiapoptotic properties, and potential to cause direct oxidative damage to host DNA by elevating the intracellular levels of reactive oxidant species in cells.[15,106] HBx activates a number of nuclear transcription pathways and can modulate cytoplasmic signal transduction pathways, including Ras-Raf-MAPK, Janus kinase/STAT (Jun kinase [JNK]/STAT), resulting in hepatic cell proliferation. In addition, HBx can directly inactivate or indirectly downregulate various tumor suppressors, such as p53, or senescence-related factors.[107] Because most of the HBV genes are extinguished in advanced tumors, the viral role in the development of HCC is most likely to be at the stage(s) of initiation or promotion of hepatocarcinogenesis. This would preclude the presence of HBx in maintaining the transformed phenotype.

Finally, HBV-associated HCC may be due to the repeated cellular division associated with the inflammatory response.[15,108] HCC associated with HBV is most common in the context of cirrhosis, although it can occur in all stages of liver disease. Cirrhosis is the result of years of inflammation and associated repair processes, during which there is considerable cell killing and repeated hepatocyte regeneration. In all types of liver damage, there is evidence of enhanced production of free radicals or significant decrease of antioxidant defense, or both.[109] Oxidative stress induced by inflammation and accumulation of viral surface proteins over a long period may give rise to high mutation rates in infected hepatocytes.

EPIDEMIOLOGY OF HEPATITIS B

Approximately 3.6% of the world's population (≈257 million people) is chronically infected with HBV, and more than 800,000 deaths due to HBV occur annually.[110,111] The prevalence of chronic HBV varies widely, ranging from 0.01% to 2% in the low-prevalence areas (United Kingdom, United States, Canada, Western Europe, and Japan), 2% to 5% in the lower intermediate areas (parts of South America, North Africa, Russia), 5% to 8% in the higher intermediate areas (South Africa, China, and Thailand), and >8% in areas of high prevalence (most of sub-Saharan Africa and a number of countries in the Western Pacific region; Fig. 145.5 and Table 145.1).The largest number of people living with chronic HBV live in the Western Pacific region (>95 million) and the African region (>75 million).[110] In some parts of Europe, typically considered a low-prevalence area, high HBV prevalence rates have been observed in persons migrating from countries with intermediate/high HBV prevalence.[112] Specific patient groups, such as injection drug users, men who have sex with men (MSM), and human immunodeficiency virus (HIV)-infected patients have been shown to have significantly higher rates of chronic HBV infection than the general population.[113] The risk of chronic infection is inversely proportional to age of acquisition of infection. In areas of high seroprevalence, HBV is more likely to be acquired perinatally, with an attendant high risk of chronic infection of 90%. For infections acquired between the ages of 6 months and 5 years, the risk of chronic infection is between 20% and 60%, and for infections acquired by immunocompetent adults the risk is approximately 5%.[114]

The epidemiology of HBV is changing with the advent of universal vaccination programs adopted by many countries. Over the past 40 to 50 years decreases in HBV prevalence in countries of South-East Asia, the Western Pacific, and the Eastern Mediterranean have been observed, whereas increases have been observed in parts of Eastern Europe and Africa (Nigeria) (8.55%–9.8%). In the United States the estimated number of new symptomatic infections per year was more than 10 per 100,000 in the mid-1980s, whereas after the adoption of universal vaccination for infants and "catch-up" vaccination for older children in 1991, this number was estimated to be about 1.1 per 100,000 in 2015.[115] The overall incidence of acute hepatitis B has declined in all age groups over this period, although it remains highest in persons age 30 to 39 years (2.6 cases/100,000 population) (Fig. 145.6).[115] Despite these declines, there are still between an estimated 850,000 and 2.2 million HBV carriers in the United States, of whom about 25% will develop serious sequelae during their lifetime, and 1800 will die annually due to complications of chronic liver disease, including HCC.[115,116]

ROUTES OF TRANSMISSION

Hepatitis B replicates to high titer in the blood (10^8–10^{10} virions/mL), especially during the acute phase of illness. Any parenteral or mucosal exposure to infected blood thus represents a potential risk for acquisition of hepatitis B and accounts for the 100 times more efficient transmission of HBV compared with HIV after needlestick exposure.[117] HBV is also found in other body fluids to a variable degree, including semen, saliva, cervical secretions, and tears, and can survive up to 7 days on environmental surfaces.[117a] Thus exposure to even minute amounts of blood or contaminated secretions may transmit virus, and infection can occur in settings of prolonged, close personal contact, such as occurs between children, or among residents of institutions for the developmentally disabled, probably due to inapparent contact of infected secretions with nonintact skin. HBV is not found in urine, sweat, or stools. The typical mode of transmission of HBV varies in part with the prevalence of infection (see Table 145.1). In high- and intermediate-endemicity areas, such as sub-Saharan Africa and South East Asia, the predominant route of transmission is perinatal or horizontal during childhood (i.e., close contact excluding parenteral, sexual, or perinatal exposure), whereas in more low-prevalence areas, transmission occurs via injection drug use and high-risk sexual behaviors. Persons at increased risk of acquiring HBV infection include members of the following groups: illicit drug users (injecting, inhaling, snorting, pill popping), heterosexual men and women and MSM with multiple partners, household contacts and sexual partners of HBV carriers, infants born to HBV-infected mothers, patients and staff in custodial institutions for the developmentally disabled, recipients of certain plasma-derived products (including patients with congenital coagulation defects) before 1992, hemodialysis patients, health and public safety workers who have contact with blood, travelers

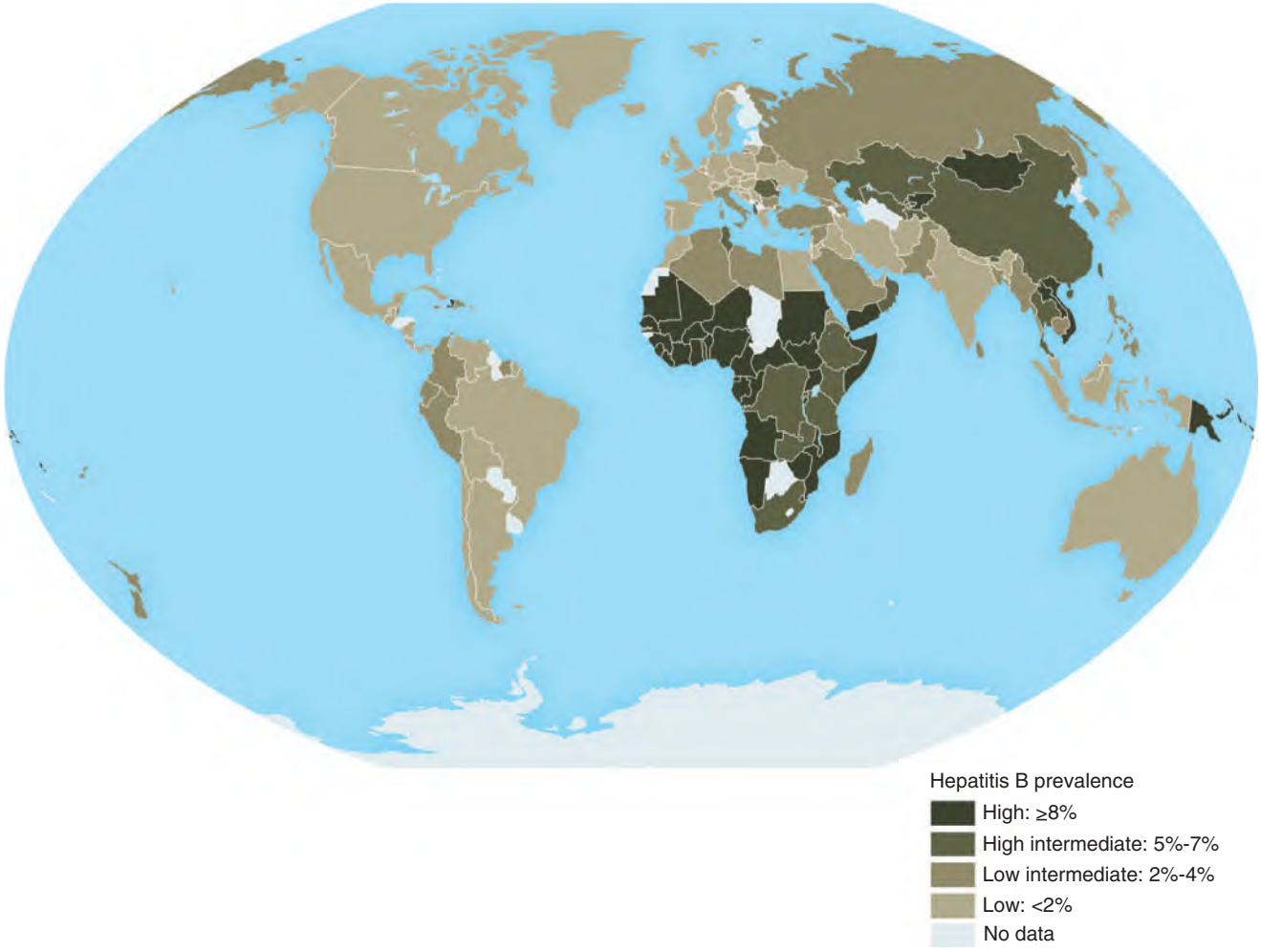

FIG. 145.5 Global prevalence of hepatitis B surface antigenemia. *(From Schweitzer A, Horn J, Mikolajczyk RT, et al. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013.* Lancet. *2015;386:1546–1555.)*

TABLE 145.1 Global Seroprevalence Rates and Modes of Transmission of Hepatitis B

CHARACTERISTIC	HIGH	INTERMEDIATE	LOW
Carrier rate (%)	≥8	2–7.99	<2
Distribution	Alaskan Eskimos, sub-Saharan Africa, Somalia, Yemen, Djibouti Haiti, Kyrgyzstan, Papua New Guinea, Solomon Islands, Nauru, Vietnam,	Eastern and southern Europe except Kyrgyzstan, Eastern Mediterranean, Western Pacific region except Australia, Japan, Papua New Guinea, Vietnam, Southeast and Central Asia, except Nepal and India	United States, Canada, Western Europe, Australia, Mexico, South and Central America except Peru, Suriname, Columbia and Belize; Nepal, India; Japan
Age at infection	Perinatal and early childhood	Childhood	Adult
Mode of transmission	Maternal and perinatal	Percutaneous, horizontal	Sexual, percutaneous

to regions with intermediate or high rates of HBV (≥2%), and persons born in areas of high HBV endemicity and their children. Persons at risk of exposure to HBV should be screened for markers of HBV infection and receive hepatitis B vaccine if seronegative (see "Prevention of Hepatitis B Virus Infection" later).

CLINICAL MANIFESTATIONS AND PROGNOSIS

The spectrum of clinical manifestations of HBV infection varies in both acute and chronic infection. During the acute phase, manifestations range from subclinical or anicteric hepatitis to icteric hepatitis and, in some cases, fulminant hepatitis. During the chronic phase, manifestations range from an asymptomatic carrier state to the signs and symptoms of cirrhosis and HCC. Extrahepatic manifestations can also occur with both acute and chronic infection.

Acute Hepatitis B

After exposure to HBV, there is an incubation period of 1 to 4 months.[118] Acute hepatitis B is a clinical syndrome indistinguishable from other acute hepatitides and often consists of a flulike syndrome with malaise, fatigue, anorexia, nausea, vomiting, and right upper quadrant discomfort. Serum sickness–like manifestations may be present before the onset of jaundice. Physical signs include jaundice (present in almost all patients) and tender hepatomegaly. The likelihood of developing icteric illness is inversely proportional to age. Symptomatic hepatitis rarely develops in children younger than 1 year, in 10% of children younger than 5 years, and between 30% and 80% of adults.[114] Most reported cases of HBV are the result of icteric illness, but it is believed that approximately two-thirds of cases either result in subclinical illness or are only diagnosed in retrospect. The acute illness may be more severe in the setting of other coinfections, such as simultaneous acquisition of hepatitis D (see

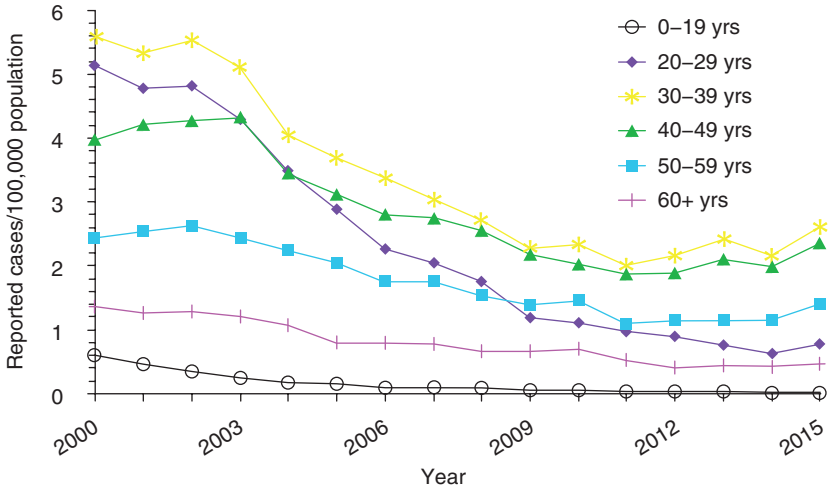

FIG. 145.6 **Incidence of symptomatic, acute hepatitis B per 100,000 population by age group: United States, 2000–2015.** *(Modified from Centers for Disease Control and Prevention.* Surveillance summaries: surveillance for viral hepatitis—United States, 2015. https://www.cdc.gov/hepatitis/statistics/2015surveillance/pdfs/2015HepSurveillanceRpt.pdf. *Accessed December 21, 2017.)*

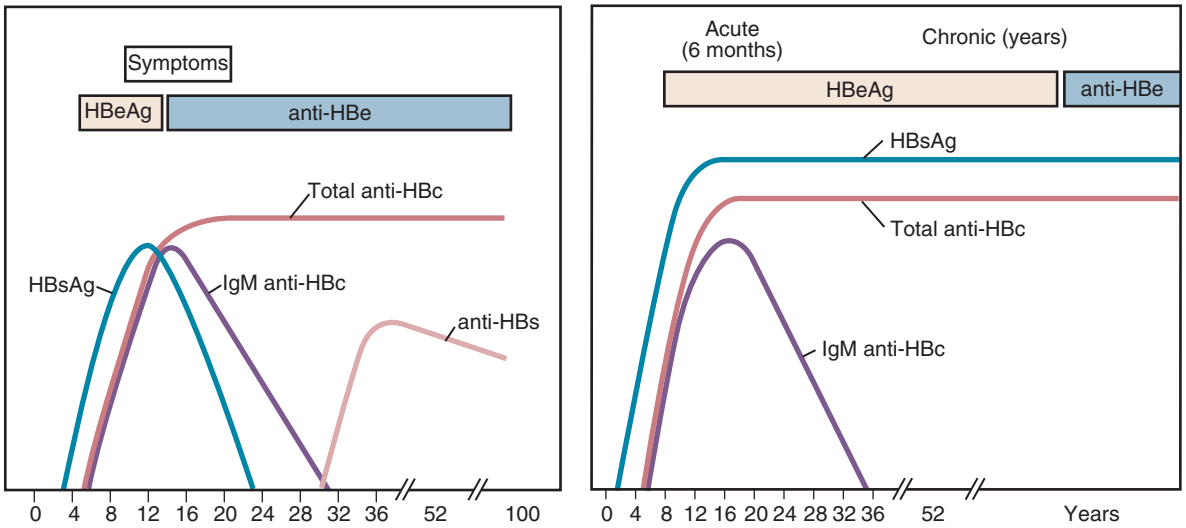

FIG. 145.7 **Typical course of hepatitis B.** *Left,* Typical course of acute hepatitis B. *Right,* Chronic hepatitis B. *HBc,* Hepatitis B core; *HBe,* hepatitis B envelope; *HBsAg,* hepatitis B surface antigen; *IgM,* immunoglobulin M.

Chapter 146), or with underlying liver disease, such as alcoholic liver disease. The symptoms and jaundice generally disappear in 1 month, but some patients have prolonged fatigue even after resolution of the elevated serum aminotransferases. See Fig. 145.7 for an illustration of a typical clinical and laboratory course of acute hepatitis B compared with that of chronic hepatitis B. In acute HBV, elevations in the concentration of ALT and aspartate aminotransferase (AST) levels with values up to 1000 to 2000 U/L can be seen, with ALT being higher than AST. The serum bilirubin concentration may be normal in patients with anicteric hepatitis. The prothrombin time (PT) is the best indicator of prognosis, with a high PT indicative of fulminant liver failure.[119] Among patients who recover, normalization of serum aminotransferases usually occurs within 1 to 4 months. Persistent elevation of serum ALT for more than 6 months indicates progression to chronic hepatitis. Rates of progression to chronic disease are similar whether or not there is symptomatic acute disease.

Acute HBV is characterized by the development of IgM antibody against the core antigen, followed by the development of immunoglobulin G (IgG) anti-HBc (see "Screening and Diagnosis of HBV Infection" later). Markers of viral replication, such as HBsAg and HBV DNA in serum, are present at the same time as anti-HBc. Loss of HBV DNA, HBeAg, HBsAg, and IgM anti-HBc and the development of anti-HBe and anti-HBs all characterize immunity. In a recent longitudinal study of Chinese patients with acute HBV, the median HBsAg clearance time from the onset of symptoms was 6 weeks, and 90% of the patients achieved HBsAg seroconversion in 19 weeks.[120] Patients who recover from acute hepatitis B are usually not cured of infection because a significant number of patients will have HBV DNA detectable by polymerase chain reaction (PCR) many years after clinical recovery.[121] They generally have lifelong protection from disease unless there is significant immunosuppression with loss of protective immune responses, such as in the setting of HIV, chemotherapy, or bone marrow transplantation.[122,123]

Fulminant Hepatitis

Fulminant hepatitis B is rare, occurring in about 1% of patients, and causes <10% of fulminant liver failure in the United States.[124,125] Patients typically present with rapidly progressive acute hepatitis <28 days from the time of symptom onset, accompanied by signs of liver failure, such as coagulopathy, encephalopathy, and cerebral edema. Mortality rates from fulminant HBV as high as 70% have been reported, although the rates have decreased substantially with advances in liver transplantation and critical care management.[126–128] Poor prognostic factors for transplant-free survival include a lower mean arterial pressure on admission and low platelet count.[124] Laboratory testing may not reveal HBsAg due to early clearance but will have IgM anti-HBc and a positive HBV DNA.

Recently, there have been increasing reports of fulminant HBV in the setting of HBV reactivation with chemotherapy or immunosuppressants, with an associated high mortality (5%–22%).[129–131] Fulminant hepatitis is also more common in the setting of coinfection with hepatitis D virus (HDV).[132]

The pathogenesis of fulminant hepatic failure is not clear but may be related to massive immune responses against the virus. Staining of the liver in fulminant HBV often reveals few copies of HBV, suggesting that it is the exuberant immune response that is primarily responsible for the pathogenesis.[133] In addition to host factors relating to immune status, several variants of HBV have also been implicated in several outbreaks of fulminant HBV, including pre-S and S gene variations; BCP; and precore and core gene variations, which result in the decrease or absence of HBeAg; *pol* gene; and *X* gene variatons.[134] Particular HBV genotypes, such as B1 and F (in the context of HDV infection), have also been shown to be associated with fulminant HBV.[135]

Chronic Hepatitis B

Chronic hepatitis B is defined by at least 6 months of persistent HBsAg. Many patients with chronic hepatitis B are not diagnosed as a result of follow-up after a case of icteric illness or due to specific symptoms, but rather as a result of incidental elevations in serum aminotransferases or due to membership in a specific risk category. Symptoms, if present, can be as nonspecific as fatigue, unless cirrhosis or HCC is present. Other less common symptoms include nausea, right upper quadrant tenderness, anorexia, myalgias, and arthralgias. Symptoms often do not correlate with severity of disease, levels of serum aminotransferases, or hepatic injury on liver biopsy. The physical examination may be normal, or there may be an enlarged liver. The presence of jaundice, splenomegaly, ascites, encephalopathy, or pedal edema suggests cirrhosis.

In patients with chronic hepatitis B, serum aminotransferases may be normal, although most patients with HBeAg-positive or HBeAg-negative chronic hepatitis B have at least mild-to-moderate elevations. Patients will have markers of viral replication, including HBsAg and often HBeAg or HBV DNA as well (see "Screening and Diagnosis of HBV Infection" later). During flares of disease activity or just before seroconversion to anti-HBe status, there may be marked elevations in the serum aminotransferases to more than 20 times normal. Flares of disease activity may be due to changes in the level of baseline HBV replication, followed by an immune response against the virus. In some patients this results in repopulation of the viral species with a new variant.[136] Patients with flares of disease activity should be followed for the development of anti-HBe, which signals control of viral replication. Cirrhosis should be suspected if there is evidence of hypersplenism, manifested as decreased platelet count, or impaired hepatic synthetic function, indicated by hypoalbuminemia, hyperbilirubinemia, or decreased albumin. Similar to the range of findings observed in other laboratory tests, findings on liver biopsy range from minimal inflammation to cirrhosis. About 2% to 5% of patients with HBV-related compensated cirrhosis develop decompensated disease each year. Decompensation can occur over time or can result from hepatitis flares.[137] Clinical signs and symptoms of decompensated disease include ascites, jaundice, hepatic encephalopathy, or variceal bleeding.

There are no characteristic pathologic findings that can distinguish hepatitis B from other forms of viral hepatitis, although liver biopsies can be stained for the presence of HBsAg and HBcAg. The most characteristic feature of chronic hepatitis B is the ground-glass hepatocyte, which is thought to be due to accumulation of HBsAg within the ER.[138] Differentiation from alcoholic liver disease can be made by characteristic patterns of steatosis, Mallory bodies, and micronodular cirrhosis, whereas chronic hepatitis C is marked by steatosis and characteristic lymphoid follicles within portal tracts. Immunosuppressed hosts may have a variant of HBV known as fibrosing cholestatic hepatitis, which reveals periportal fibrosis, hepatocyte ballooning, bile stasis, and mild or absent inflammation.[139]

Precore or HBe-Negative Mutants

Early reports described patients in whom HBeAg was absent, although HBV DNA continued to be present at a high level, typically in association with severe liver disease.[140–143] This is due to the development of HBV pre-C or core promoter mutants that cannot produce HBeAg (see "Translation" earlier).[144] The most frequent pre-C mutation is a guanine (G) to adenine (A) change at nucleotide 1986, which produces a translation stop codon at amino-acid position 28 in the pre-C region and abolishes production of HBeAg.[145] A1762T and G1764A are frequently occurring mutations that take place in the basal core promoter (BCP) region. All these mutations abrogate HBeAg synthesis without affecting the replication of the virus, which persists and results in chronic inflammation. Several large cohort and case-control studies have demonstrated an association between BCP A1762T/G1764A mutations and an increased risk of liver disease progression and HCC.[146] At the time of initial presentation, 29% to 38% of HBeAg-negative chronic hepatitis B patients have cirrhosis.[147,148] These patients have very low rates of spontaneous seroconversion of <0.5% per year[149] and are more difficult to treat.[141] Originally, HBeAg-negative hepatitis was thought to be predominantly a geographic phenomenon because it is more prevalent in certain parts of the world, especially Asia and the Mediterranean. However, studies suggest that the likelihood of HBeAg-negative chronic hepatitis B is related to duration of infection, as suggested by the older age at presentation,[147] which would make it appear to be of higher prevalence in areas where perinatal transmission predominates. HBeAg-negative disease is also more frequent with genotypes C, D, and F than with genotype A and B infection and presumably contribute to genotype specific immunopathogensis.[135,150]

Prognosis of Untreated Chronic Hepatitis B

The likelihood of morbidity and mortality in chronic hepatitis B is directly related to the development of cirrhosis. Among chronic HBsAg-positive patients, the risk of developing cirrhosis ranges from 1 to 5.4 per 100 person-years, with a 5-year cumulative probability of progression ranging from 8% to 20%.[147,151,152] For persons who clear HBsAg, the prognosis is good, although, perhaps surprisingly, not entirely benign. In one study of 189 patients who were noncirrhotic at the time of HBsAg clearance, 3 (1.6%) developed cirrhosis, 2 (1.1%) developed HCC, and 1 died of HCC. These complications all developed in patients with concurrent hepatitis C virus (HCV) or HDV infection, however.[153] In the absence of cirrhosis the long-term prognosis even of HBsAg-positive patients is good. In a 16-year follow-up study of 317 HBsAg-positive blood donors from Montreal, for example, only 3 died from HBV-related cirrhosis and none developed HCC.[154] In a US-based study of 6689 patients with a chronic hepatitis B who did not receive therapy and who were followed for 10 years, 3.1% died from HBV-related causes, with the highest percentage (15.5%) in those older than 65 years.[155] However, in another study of HBsAg-positive patients in England and Wales, during a mean of 22 years of follow-up, 17.4% of deaths were due to HCC or liver disease.[156]

Multiple variables account for the wide estimates of risk. A study from Taiwan (REVEAL [*R*isk *E*valuation of *V*iral Load *E*levation and *A*ssociated *L*iver Disease/Cancer]–HBV) of 3582 people with chronic hepatitis B followed for 11 years found that the strongest predictor of progression to cirrhosis was the HBV DNA level, which was independent of the HBeAg status, and inflammation as represented by ALT values.[157] Individuals with $>10^6$ copies/mL of HBV DNA had a 36.2% cumulative incidence of cirrhosis compared with 4.5% in those with <300 copies/mL. Most of the people in this study acquired HBV in infancy or early childhood; thus this study may not be applicable to adult-acquired HBV. The rate of progression to cirrhosis may be higher in patients who are HBeAg negative compared with those who are HBeAg positive,[141] although these findings may be confounded by a longer duration of disease in HBeAg-negative patients and presence of precore and BCP mutations.[158,159] Patients with more severe inflammation and fibrosis at the time of presentation are also more likely to progress to cirrhosis. Fattovich and colleagues[71] found that 30% of patients with moderate, chronic active hepatitis developed cirrhosis on histology after 6 years, whereas 50% of patients with severe, chronic active hepatitis (bridging necrosis) developed cirrhosis on histology after 4 years. Consumption of alcohol also increases both the risk of cirrhosis and HCC.[160] Infection with HCV, when accompanied by active replication of

both viruses, also increases the rate of progression to cirrhosis,[161,162] as does HDV.[163]

For patients with compensated cirrhosis, survival is 84% at 5 years and 68% at 10 years without treatment.[164] Once cirrhosis develops the risk of decompensation is 20% to 25% per year without treatment.[71,165] High HBV DNA levels and HBeAg seropositivity and persistence have been found to be independently associated with decompensated disease and death.[166–168] The prognosis is poor after the development of decompensation, with estimated survival rates of only 55% to 70% at 1 year and 14% to 35% at 5 years.[151,169]

Hepatocellular Carcinoma

Another major cause of mortality in chronic hepatitis B is HCC, which has a poor prognosis unless caught in a small, surgically resectable stage. HCC is the sixth most common cancer and the fourth leading cause of cancer-related deaths worldwide. An estimated 800,000 new cases occur each year, with chronic hepatitis B accounting for 18% (Europe) to 65% (China) of total cases.[170] The lifetime risk for untreated HBV chronic carriers is currently estimated to be about 20% to 40% and about 10- to 25-fold higher compared with non–HBV-infected controls.[171,172] In a recent systematic review of 66 studies of HCC in HBV-infected persons from Europe and North America, summary estimates of incidence rates according to liver disease status (95% confidence intervals [CIs]) were 0.05 (0.03 to 0.08), 0.31 (0.22 to 0.41), 0.42 (0.27 to 0.56), and 2.97 (2.35 to 3.59) in inactive carriers, asymptomatic carriers, subjects with chronic hepatitis, and with compensated cirrhosis, respectively.[173] Studies suggest that the interval between acquisition of HBV and development of HCC spans several decades (average, 30 years).[174] However, that interval is much shorter in parts of sub-Saharan Africa, where 43% of HBV-related HCC has been shown to develop in adults younger than 40 years.[174a] Coinfection with HDV, HCV, or HIV augments the risk of HCC development in HBV infection. More recently, patients with genotype C (Asians) and A1 (Africans),[175] as well as those with the A1762T and G1764A basal core promoter and pre-S gene mutations, have been shown to have a higher risk of HCC.[135,176] Other risk factors include older age, male gender, heavy alcohol consumption, metabolic syndrome, exposure to aflatoxin B, and family history of HCC.[177–180] Levels of HBV DNA are also a risk factor, with levels $>10^5$ copies/mL (≈20,000 IU/mL) being strongly linked to the development of HCC.[177] In a recent study the importance of serum HBV DNA level as a predictor of HCC was even greater in HBeAg-negative patients with normal ALTs and no cirrhosis at baseline.[181] HBsAg levels have also been shown to positively correlate with the development of HCC and better predict HCC in HBeAg-negative patients with low HBV DNA. In the Taiwanese *E*lucidation of *R*isk F*a*ctors for *Di*sease *C*ontrol or *A*dvancement in *T*aiwanese H*e*patitis *B* Carriers (ERADICATE-B) study, among a subset of 1068 HBeAg-negative individuals with HBV DNA levels ≥2000 IU/mL, the risk of HCC was significantly increased in these patients with an HBsAg level ≤1000 IU/mL compared with those with a level <1000 IU/mL (hazard ratio, 5.4; 95% CI, 2.1 to 14.2).[182] Clearance of HBsAg by the age of 50 years appears to decrease the risk of HCC.[183] Finally, treatment with nucleoside analogues decreases the risk of HCC about 30% to 80%.[180] To date, no studies have shown convincing data for an association between occult HBV, defined as the presence of HBV DNA without serologic markers of HBV infection, and the presence of HCC.

Extrahepatic Manifestations of Hepatitis B

Extrahepatic manifestations can occur in approximately 20% of patients with acute and chronic hepatitis B and are thought to be mediated by circulating immune complexes. Acute hepatitis may be manifested in 10% to 20% of patients as a serum sickness–like illness with fever, skin rash, arthralgias, and polyarthritis, typically occurring just before the onset and subsiding with the development of jaundice. The skin rash can be of virtually any type, including erythematous, macular, maculopapular, urticarial, or petechial. The most common extrahepatic clinical manifestations of chronic hepatitis B include sensorimotor neuropathies, myalgias, arthralgias, Sjögren syndrome, glomerulonephritis, uveitis, and Raynaud syndrome.[184] A number of autoantibodies can also be seen, including cryoglobulinemia and rheumatoid factor. Polyarteritis nodosa (PAN) is a rare complication of chronic hepatitis B, which usually manifests within the first 6 months of infection. It is a vasculitis of small- to medium-size arteries and typically presents with fever, rash, hypertension, eosinophilia, abdominal pain, renal disease, and polyarthritis. In one review the frequency of HBV infection in PAN cases was 33.7%; however, the overall incidence is declining.[185] Treatment of PAN usually includes the combination of plasma exchanges, anti-HBV therapies, and a short course of steroids in cases of life-threatening organ involvement.[186–188] Recovery from vasculitis can occur in up to 81% when HBeAg seroconversion and decreased viral replication are achieved.[189] HBV-related cryoglobulinemic vasculitis has been successfully treated with nucleoside therapies.[190,191] Glomerular disease also occurs as a manifestation of HBV infection. Nephrotic syndrome secondary to membranous or membranoproliferative glomerulonephritis is a rare complication of HBV infection occurring predominantly in children with active viral replication. The typical presentation is with nephrotic-range proteinuria. Approximately 30% to 60% of children with HBV-related membranous nephropathy undergo spontaneous remission, usually in association with HBeAg to anti-HBe seroconversion.[192] Progression to renal failure can occur, particularly in adults. The prognosis of HBV-related membranous nephropathy is variable. In adults it may lead to progressive renal insufficiency[193]; however, it can also be successfully treated. In a recent meta-analysis of 82 patients, treatment with IFN-α (five studies) or lamivudine (one study) resulted in proteinuria remission in 65.2% of the patients.[194]

CLINICAL MANIFESTATIONS AND NATURAL HISTORY IN SPECIAL HOSTS

Individuals With Human Immunodeficiency Virus Infection

Because of common parenteral routes of transmission, HBV and HIV are frequently seen in concert. Since the introduction of antiretroviral therapies (ARTs), the incidence of acute HBV infection in persons living with HIV has been declining. Earlier studies reported the incidence of HBV as high as 12.2 cases per 1000 person-years, although lower in those receiving either ART with lamivudine, ART without lamivudine, or one or more doses of HBV vaccine.[195] In a recent study of 2375 HBV-uninfected MSM enrolled in the Multicenter AIDS Cohort Study, the overall unadjusted HBV incidence rate was 9.6 per 1000 person-years. Incidence rates were significantly lower in the highly active antiretroviral therapy (HAART) era than in the pre-HAART era among both those infected with HIV (incidence rate ratio (IRR), 0.2 [95% CI, 0.1 to 0.4]) and not infected with HIV (IRR, 0.3 [95% CI, 0.2 to 0.4]).[196] Of interest, this study also found that receiving effective ART (HIV RNA < 400 copies/mL) was protective against acute hepatitis B, whereas men receiving ART who had HIV RNA ≥400 copies/mL were not protected. In fact, the incidence rate of acute hepatitis B in HIV-infected men with effective ART was equivalent to HIV-uninfected men.[196] Current estimates are that between 40% and 60% of individuals who are HIV seropositive in the United States are positive for some marker of past HBV infection, and 4% to 10% are HBsAg positive.[197] In the regions with high HBV endemicity, up to 25% of HIV-infected persons are coinfected with chronic HBV.[198] Patients with HIV and acquired immunodeficiency syndrome are more likely to have chronic HBV infection, increased replication manifested as higher HBV DNA, and increased likelihood of HBeAg positivity.[199] They are also more likely to have occult HBV; therefore HIV patients should be screened for both HBsAg and anti-HBc. The availability of ARTs with activity against both HIV and HBV has resulted in improved outcomes for patients with HIV/HBV coinfection. However, the risk of liver disease and overall mortality remains higher compared with those with either infection alone, even with effective virologic suppression.[200–202] In fact, as ART has reduced HIV-related deaths, liver-related deaths have now emerged as one of the leading causes of deaths in persons living with HIV. Many of these liver-related deaths are due to HCC, which among HIV/HBV coinfected persons is approximately fivefold to sixfold higher than in the general population, although less in the era of ART.[203] Other factors likely contribute to this increased risk of HCC, such as obesity, age,

HCV coinfection, and metabolic factors. Lower CD4 counts in HIV-HBV coinfected persons have also been associated with high HBV DNA levels and an increased risk of HCC and mortality.[204–206] Finally, isolated anti-HBc is found in approximately 16% to 20% of HIV-infected patients.[207] One study demonstrated that the isolated anti-HBc serologic pattern is usually stable, but when changes from isolated anti-HBc occur, the transition is usually to anti-HBc and anti-HBs positivity, suggesting that isolated anti-HBc is due to intermittently low levels of anti-HBs.[207] Rates of occult hepatitis B in those with isolated anti-HBc are variable, ranging from <5% up to 90%.[207–210]

Hepatitis B After Liver Transplantation

Reinfection of the allograft accounts for the majority of cases of HBV after liver transplantation in patients with chronic HBV. HBV can also be acquired de novo in patients who are HBsAg negative from anti-HBc–positive donors, with rates of infection as high as 77% in the absence of HBIG or antiviral prophylaxis.[211–213] This includes previously vaccinated recipients in whom HBV acquisition can also occur.[214,215] Recipients who are anti-HBs positive and anti-HBc positive are at the least risk of de novo infection occurring in 0% to 4%. Reactivation of HBV has also been reported in recipients who are HBsAg negative and anti-HBc positive pretransplant. Before the use of prophylactic HBIG and nucelos(t)ide therapy at the time of transplantation, graft reinfection rates approached 80% to 100% and was a major cause of allograft dysfunction, cirrhosis, and graft failure.[216] Reinfection was strongly correlated with high levels of HBV DNA, antiviral resistance, and HBeAg seropositivity.[217] The use of HBIG and nucleos(t)ide therapy, considered the standard of care in transplantation programs, has resulted in significant reductions in recurrence of HBV infection in the allograft, and post-transplant survival has improved markedly.[218] In a large study conducted between 1988 and 2002, the 1-, 5-, and 10-year patient survival rates were 91%, 81%, and 73%,[219] respectively, which are comparable with other conditions leading to transplantation. Specific HBV therapies used in liver transplantation are discussed in more detail under "Management in Special Populations" and "Liver Transplantation."

Hepatitis B After Other Types of Transplantation

HBV is associated with high morbidity and mortality in renal transplant recipients, which is thought to be due to persistent viral replication and reduced HBsAg seroconversion as a result of the effects of immunosuppression. HBV reactivation has occurred in renal transplant patients with previously resolved HBV infection characterized by negative HBsAg and positive anti-HBc, with or without anti-HBs, but the overall risk is low.[220] Reactivation is higher among those without anti-HBs compared with those with anti-HBs.[221] All renal transplant recipients who are HBsAg positive should receive antiviral therapy with nucleos(t)ide therapy (entecavir or tenofovir alafenamide [TAF] preferred) before or at the time of transplantation and continue indefinitely. Renal transplant recipients with anti-HBc with or without anti-HBs should have HBV DNA measured every 3 to 6 months after renal transplantation and receive nucleos(t)ide therapy if viral reactivation occurs but before onset of clinical hepatitis.[222] The risk of de novo infection in HBV naïve kidney recipients depends on use of prophylaxis and HBV immunity in the recipient and ranges from 0% to 27%. HBV reactivation is low among patients receiving transplants from anti-HBc–positive donors (1%–4%) and may present subclinically.[223] HBV prophylaxis before transplantation protects against HBV acquisition in nonimmune renal transplant recipients whose donors are anti-HBc positive. Recipients of bone marrow transplantations are also at risk of hepatitis B reactivation, which may present as a severe flare at the time of withdrawal of immunosuppression[224] or as progressive chronic liver disease.[225] HBV reactivation has been reported in 10% to 21% of allogeneic hematopoietic stem cell transplantation (HSCT) recipients with resolved HBV infection and without antiviral prophylaxis and 45% in HBsAg-positive auto-HSCT patients.[226,227] Lamivudine in these patients has been shown to reduce the incidence and severity of HBV reactivation.[228,229] Of interest, there have been case reports of hepatitis B cure after bone marrow transplantation due to the transfer of HBV-specific immune cells in the graft.[230,231]

Coinfection With Hepatitis C

Rates of HCV coinfection among patients with HBV range from 3% to 18%, depending on the geographic area and the epidemiologic risk group.[232] HCV infection is especially frequent in HBV endemic areas, in intravenous (IV) drug users, and among persons infected with HIV.[233] Superinfection and acute infection with HCV can result in suppression of HBV replication and clearance of HBsAg and HBeAg.[75,234–236] In turn, inhibition of HCV replication has been observed in patients with chronic HCV superinfected with HBV and higher rates of HCV RNA clearance have been reported in HBV/HCV coinfected compared with HCV monoinfected individuals.[237,238] However, when both viruses are replicating, the liver disease is usually more severe than in patients infected by HBV or HCV alone.[239–241] Patients with dual HBV and HCV infection may also have a higher rate of HCC compared with patients infected by either virus alone.[242] Treatment of HCV in HCV-HBV coinfected individuals has resulted in significant declines in HCC, all-cause mortality, and liver-related mortality.[243] In the past, treatment of HCV with PEG IFN was associated with both relatively high rates of HBsAg seroclearance (≈11%) and HBV reactivation (≈62%).[244,245] HBV reactivation has also been demonstrated in HBV-HCV coinfected patients during and after HCV treatment, with directly acting antivirals (DAA) in patients who are HBsAg positive with undetectable or low HBV DNA and in patients who are isolated anti-HBc positive.[246,247,248] The risk of reactivation is highest among HBsAg-positive individuals; reactivation among patients with isolated anti-HBc is limited to case reports.[249] Current Infectious Diseases Society of America/American Association for the Study of Liver Disease (AASLD) guidelines recommend all patients initiating HCV DAA therapy should be assessed for HBV coinfection with HBsAg testing and for evidence of prior infection with anti-HBs and anti-HBc. In HBsAg-positive patients HBV therapy should be initiated for patients who meet criteria for treatment (see "Treatment"); for patients whose HBV DNA level is below treatment initiation criteria, either prophylactic antiviral therapy or monitoring of HBV DNA levels during and immediately after DAA therapy with preemptive therapy is recommended.[250]

SCREENING AND DIAGNOSIS OF HBV INFECTION

Infection with HBV is associated with characteristic patterns of hepatitis B antigens and antibodies. In addition to confirming the stage of HBV infection, proper interpretation of the available tests will aid in the monitoring of patients and selection for antiviral therapy.

Acute Hepatitis

The diagnosis of acute hepatitis B is based on the detection of HBsAg and IgM anti-HBc (Table 145.2; see Fig. 145.7). During the replicative phase of infection, HBeAg and HBV DNA are also present. Recovery is accompanied by the disappearance of markers of HBV replication and the appearance of antibodies to these proteins.

HBsAg is the serologic hallmark of HBV infection and is the first marker to appear after acute infection. HBsAg appears in serum 1 to 10 weeks after an acute exposure to HBV, before the onset of symptoms or elevation of serum ALT. In patients who subsequently recover, HBsAg usually becomes undetectable after 4 to 6 months, followed by development of anti-HBs, as early as 8 months after infection. In most immunocompetent patients who recover, anti-HBs persists for life, thereby conferring long-term immunity.

HBcAg is an intracellular antigen that is not detectable in serum. Anti-HBc can be detected throughout the course of HBV infection, and its presence signifies natural infection. During acute infection, anti-HBc is detectable at the time symptoms appear (9–21 weeks after exposure) and is predominantly IgM class. It can be the sole marker of HBV infection during what is called the *window period* between the disappearance of HBsAg and the appearance of anti-HBs. Although IgM anti-HBc is usually an indicator of acute HBV infection, it may remain detectable up to 2 years after the acute infection and can be detectable during HBV flares. IgG anti-HBc persists even in individuals with HBs antigenemia, indicating that the presence of this antibody does not confer protection against viral replication.

TABLE 145.2 Interpretation of Serologic Tests in Hepatitis B

TEST	ACUTE HEPATITIS B	IMMUNITY THROUGH INFECTION[a]	IMMUNITY THROUGH VACCINATION	CHRONIC HEPATITIS B
HBsAg	+	–	–	+
Anti-HBs	–	+	+	–
HBeAg	+	–	–	±[b]
Anti-HBe	–	±	–	±
Anti-HBc	+	+	–	+
IgM anti-HBc	+	–	–	–
HBV DNA	+	–	–	+
ALT	Elevated	Normal	Normal	Normal or elevated

[a]On occasion, individuals with past infection have isolated anti-HBc only. The presence of an isolated immunoglobulin G anti-HBc may indicate a window period during acute infection or remote prior infection with loss of HBsAg or anti-HBs. In such cases an HBV DNA test may prove useful.
[b]Chronic hepatitis B with a precore mutant is $HBeAg^-$ and anti-HBe^+.
ALT, Alanine aminotransferase; *anti-HBc,* antibody to hepatitis B core antigen; *anti-HBe,* antibody to hepatitis B e antigen; *anti-HBs,* antibody to hepatitis B surface antigen; *DNA,* deoxyribonucleic acid; *HBeAg,* hepatitis B e antigen; *HBsAg,* hepatitis B surface antigen; *HBV,* hepatitis B virus; *IgM,* immunoglobulin M.

Past Hepatitis B Infection and Vaccination

Anti-HBs is the protective antibody and is present both after recovery from acute infection and successful vaccination. These two can be distinguished by testing for total anti-HBc (Table 145.3). Recovery from an HBV infection is characterized by the presence of both anti-HBs and total anti-HBc. Immunity to HBV after vaccination is indicated by the presence of anti-HBs only.

Chronic Hepatitis B Infection

The diagnosis of chronic hepatitis B is based on the persistence of HBsAg for more than 6 months. Additional tests for HBV replication—HBeAg and serum HBV DNA—should be performed in those with chronic hepatitis B to further classify the disease and to determine the need for treatment. In adults there are two groups of patients with chronic hepatitis B who do not need treatment but who should be monitored. The first are those who are HBeAg negative with an undetectable HBV DNA and normal serum aminotransferases. These patients are in the inactive phase of chronic hepatitis B (previously known as "inactive carriers") and should have aminotransferases tested every 6 to 12 months to detect reactivation. The annual rate of reactivation is about 4%, with pre-C mutations, male sex, and age older than 30 years being predictors of reactivation.[251] The second group is patients who are in the immune-tolerant phase of infection (see "Natural History" earlier) with HBeAg and high HBV DNA levels but normal or minimally elevated serum aminotransferases. They should also be monitored every 6 to 12 months with aminotransferase testing.

Those with progressive liver disease who should be considered for treatment can be either HBeAg negative or positive, have detectable HBV DNA, and usually have elevated serum aminotransferase concentrations. The HBV DNA level in HBeAg-positive chronic hepatitis B patients tends to be higher than in those with HBeAg-negative chronic hepatitis B.

In HBeAg-positive patients, development of anti-HBe can occur at any point during chronic hepatitis B infection; it occurs spontaneously in a small percentage of patients per year. It is often accompanied by an increase in serum aminotransferases, followed by a disappearance of HBeAg and HBV DNA from the serum and improvement in liver inflammation. However, some patients continue to have active liver disease and detectable HBV DNA in serum after HBeAg seroconversion.[252]

Measures of Hepatitis B Replication

PCR-based assays are the standard way to quantify HBV replication. Several real-time PCR assays are available now and have lower limits of sensitivity of approximately 10 IU/mL and upper limits of 4×10^9

TABLE 145.3 Selection of Patients for Treatment in Chronic Hepatitis B

HBeAg	HBV DNA[a]	ALT[b]	TREATMENT STRATEGY
+	>20,000 IU/mL	<2 × ULN	Observe patient Consider liver biopsy or noninvasive measurement of liver disease if age > 40, ALT > 1 but <2 × ULN, family history HCC, HIV positive; treat if moderate-to-severe inflammation or fibrosis
+	>20,000 IU/mL	>2 × ULN	Observe 3–6 mo for spontaneous HBeAg seroconversion before treatment Treatment with PEG IFN-α (48 wk) or NUC (indefinite in most; minimum 6–12 mo after HBeAg seroconversion)
–	>20,000 IU/mL	>2 × ULN	Treatment with NUC (end point not defined; likely indefinite) PEG IFN-α (48 wk) second line
–	>2000 IU/mL	1 to <2 × ULN	Consider liver biopsy or noninvasive measurement of liver disease and treatment if moderate-to-severe inflammation or fibrosis
–	>2000 IU/mL	<ULN	Observe; treat if HBV DNA or ALT increase
+/–	+	Cirrhosis	Compensated: treat with NUC if HBV DNA detectable Decompensated: NUC; consider liver transplantation
+/–	–	Cirrhosis	Compensated: observe Decompensated: consider liver transplantation

[a]Conversion factor to copies/mL = 5.6 (20,000 IU/mL is approximately 10^5 copies/mL).
[b]Also use moderate-to-severe necroinflammation on liver biopsy as guide.
ALT, Alanine aminotransferase; *anti-HBe,* antibody to hepatitis e antigen; *DNA,* deoxyribonucleic acid; *HBeAg,* hepatitis B e antigen; *HBV,* hepatitis B virus; *HCC,* hepatocellular carcinoma; *HIV,* human immunodeficiency virus; *NUC,* nucleoside or nucleotide; *PEG IFN-α,* pegylated interferon-α; *ULN,* upper limit of normal.
Modified from Terrault N, Bzowej NH, Chang KM, et al. AASLD Guidelines for the treatment of chronic hepatitis B. Hepatology. *2016;63;261–283.*

IU/mL. The major role of HBV DNA assays is to establish the phase of the infection and assess candidacy for treatment. In those who are treated, monitoring HBV DNA is essential for assessing the response. HBV DNA is also useful in several other circumstances. The first is distinguishing the window period of acute infection from chronic infection in those patients who are IgG anti-HBc only. HBV DNA is also useful in cases of fulminant hepatitis, where there may be undetectable levels of HBsAg on presentation. HBV DNA testing is also indicated in patients in whom there may be a false-negative HBsAg test, which occurs due to mutants in the surface protein that are not detected by all HBsAg assays.[253]

The enhanced sensitivity of the newer tests, especially PCR-based methods, has also raised questions about the significance of low-level viremia. Recovery from acute infection was formerly thought to be accompanied by complete clearance of HBV from serum. However, use of PCR-based methods has demonstrated that some individuals may have very low-level replication in the absence of any biochemical or histologic markers of liver injury.[254] The significance of this is not known, although some studies have suggested that such "occult" hepatitis B is associated with progressive liver disease. The threshold level that is associated with a risk of progression of chronic liver disease is not known. An arbitrary value of ≥20,000 IU/mL or 10^5 copies/mL has been suggested as a diagnostic criterion for chronic hepatitis,[254] but lower levels may be seen in patients with ongoing liver injury, especially in HBeAg-negative patients. Because levels of HBV DNA may fluctuate over time in a given individual, the use of HBV DNA testing as a sole determination of future prognosis is not recommended.[255] Recently there has been increasing interest in alternative biomarkers of HBV infection, such as hepatitis B core-related antigen (HBcrAg) or HBV RNA, that may better define the activity of chronic HBV infection, monitor infection, and predict long-term outcomes.[256–258] However, more data are needed on these markers before they can be incorporated in routine clinical care.

Isolated anti-HBc

The isolated presence of anti-HBc without other HBV serologic markers is not uncommon, being present in 0.4% to 2.3% of blood donors in low-prevalence areas[259,260] and in 10% to 20% of the population in endemic countries.[261] Isolated anti-HBc can be found in patients during the window period of acute hepatitis, many years after resolution of acute hepatitis due to a decline of anti-HBs to undetectable titers, or rarely, after years of chronic infection with decline of HBsAg to titers below the limit of detection. Individuals with isolated anti-HBc should have repeated testing done to exclude false-positive results and IgM anti-HBc to exclude acute hepatitis if that is suspected. If there are unexplained aminotransferase elevations in a patient with isolated anti-HBc, HBV DNA testing is recommended. Isolated anti-HBc is more common in people coinfected with HIV and in those with both chronic and cleared HCV.[207] The clinical significance of isolated anti-HBc with or without a low-level positive HBV DNA test is not clear; however, such patients should be considered potentially infectious because transmission of HBV from blood and organ donors with anti-HBc alone has been reported.[262,263]

MANAGEMENT OF HEPATITIS B

Chronic Hepatitis B

The goals of antiviral therapy in hepatitis B are reduction of the morbidity and mortality due to liver disease. The beneficial effects of antiviral therapy on reducing the incidence of cirrhosis and HCC have been shown for all of the approved anti-HBV medications. In a meta-analysis the risk reduction for cirrhosis with antiviral treatment was 0.39 (95% CI; 0.20 to 0.75) in observational studies and was 0.55 (95% CI; 0.38 to 0.78) in randomized clinical trials.[250] Reductions in death, HCC, and hepatic decompensation were also demonstrated.

Because reduction in morbidity and mortality is challenging to study, a variety of end points have been used as surrogates to define response to treatment in clinical trials, including normalization of serum aminotransferases (biochemical response), improvement in liver histology (histologic responses), achieving an undetectable HBV DNA in the serum (virologic response), and loss of HBeAg with or without anti-HBe (serologic response).[250] Elimination of HBsAg is rare but important because the risk of HCC continues to be elevated above baseline among patients with persistent HBs antigenemia.[183] The rate of HBsAg loss with IFN or tenofovir disoproxil fumarate (TDF) alone after 48 weeks of treatment is in the range of 3% to 4%.[36,264,265] Because long-term studies have demonstrated that either seroconversion to anti-HBe or durable suppression of HBV DNA is associated with reduction in cirrhosis,[250,266–268] an improvement in survival,[269–272] and reduction of the rates of HCC,[273] these are the most common surrogate markers used in both clinical practice and clinical trials to monitor the efficacy of therapy. Quantitative HBeAg and HBsAg have also been studied, but they are not clearly superior to the existing markers in terms of predicting long-term outcome. Markers such as circulating HBcrAg and pregenomic RNA are being assessed as additional potential markers (see "Measures of Hepatitis B Replication").

Acute Hepatitis B

Treatment in acute hepatitis B is generally supportive. Medication lists should be reviewed, and patients should be reminded to avoid medications metabolized by the liver if possible or limit the doses. This is particularly true for agents such as acetaminophen, which patients may be taking to minimize discomfort and fever. Treatment of fulminant hepatitis is also supportive, including liver transplantation for those patients who do not appear to have spontaneous recovery. Treatment with antiviral agents is not indicated for acute hepatitis B because approximately 95% will spontaneously seroconvert to anti-HBs; however, there is some evidence of benefit for cases of acute fulminant HBV. One randomized controlled trial of lamivudine versus placebo in acute severe hepatitis B showed greater decline in HBV DNA at 4 weeks with lamivudine but did not show long-term clinical benefit.[274] Several case series support that lamivudine, entecavir, and TDF may be beneficial when given before the onset of liver failure.[275,276] TAF should also be effective, but it has not been studied in fulminant, acute hepatitis B. If given, treatment for fulminant hepatitis B should be continued for 3 months after HBsAg seroconversion or for at least 12 months after HBeAg seroconversion if HBsAg loss does not occur. Treatment is also recommended for protracted severe acute hepatitis when increase in international normalized ratio and jaundice last for more than 4 weeks.[250]

Selection of Patients for Treatment

(Also see Chapter 117.)

The currently available treatments all have limited short- and long-term efficacy, as well as substantial costs, both in terms of side effects (e.g., as in the case of IFN) and financial considerations. Thus not all patients warrant treatment. Current recommendations are to treat all cirrhotic patients with detectable HBV DNA. For noncirrhotic patients, treatment should be initiated with detectable HBV DNA—either HBeAg positive with a HBV DNA >20,000 IU/mL or, in the case of HBeAg-negative disease, with HBV DNA >2000 IU/mL—and evidence of liver disease (serum aminotransferases greater than two times the ULN [30 U/L for men and 19 U/L for women] or moderate-to-severe necroinflammation on biopsy) (see Table 145.3).[250] These HBV DNA cutoffs were initially arbitrary, but there is evidence that at these levels the risk of progressive liver disease and HCC increases.[157] Because HBV DNA levels can fluctuate, serial monitoring of levels is important if treatment is not initiated.

Interferon

IFN-α is the recombinant version of one or more proteins that are naturally produced by the body in response to viral infection and has been used in the treatment of hepatitis B for more than 25 years. The precise mechanism of action as an anti-HBV therapeutic has not been established. One study demonstrated that IFN-α decreases transcription of pregenomic RNA and subgenomic RNA from cccDNA.[277] PEG IFN-α, given as a once-a-week injection of 180 μg, has supplanted standard IFN-α for treatment of hepatitis B. Factors predictive of a high response to PEG IFN-α are low HBV DNA, aminotransferase elevation greater than twice the ULN, age, female sex, and genotype A or B (compared with genotype C or D).[278] There is also some evidence that IL28B genotype may be associated with response to PEG IFN-α.[279] Treatment of patients

with normal ALT results in response rates of <10%,[280] suggesting an important contribution of the immune response in IFN-based therapy.

PEG IFN-α is administered as subcutaneous injections. Patients should be treated for at least 48 weeks, and there is some evidence that up to 72 weeks will increase the rate of sustained response in those with HBeAg-negative disease.[281] In HBeAg-positive patients, 1-year rates of HBeAg seroconversion are 27%[36,278]; the durability of the response is improved if PEG IFN-α is continued for at least 24 weeks after seroconversion. Quantitative HBeAg is a predictor of response to PEG IFN-α in HBeAg-positive disease, with levels >20,000 IU/mL at 24 weeks being associated with decreased likelihood of HBeAg seroconversion.[282] If HBeAg or HBsAg loss occur, the durability of the response in one study of 172 HBeAg-positive patients followed for 3 years was 81% and 30%, for HBeAg and HBsAg, respectively.[283] In HBeAg-negative disease, the rate of sustained undetectable HBV DNA, measured 24 weeks after 48 weeks of treatment, is approximately 19%. Given the toxicity of IFN-α, most providers do not use it in HBeAg-negative disease as the tolerability of long-term nucleos(t)ides is better.

Acute side effects of PEG IFN-α therapy include flulike illness, fever, myalgias, headache, and fatigue. Other side effects seen after prolonged dosing include leukopenia and thrombocytopenia, hair loss, and changes in mood, including depression, which can be severe. IFN therapy can also lead to the development of autoantibodies, such as antithyroid antibodies, and worsening of other autoimmune disorders.[284] PEG IFN-α is contraindicated in decompensated cirrhosis because it may precipitate flares with subsequent decompensation.[285]

Nucleoside and Nucleotide Analogues

Recognition that HBV, like HIV, has an RT step in its life cycle led to the testing of many nucleoside agents (Table 145.4). Because these agents are better tolerated than IFN-α, they are used long term for both viral suppression and improvement in histologic disease. Unlike IFN-α, nucleos(t)ide agents can be used in the setting of hepatic decompensation[286] and may even prevent or delay the need for transplantation.[287,288] However, resistance to some of these agents develops over time (see "Viral Resistance" later). The currently approved agents in this category include lamivudine, adefovir dipivoxil, telbivudine, entecavir, TDF, and TAF (also see Chapter 47). Emtricitabine, which is coformulated with TDF and TAF as an anti-HIV therapeutic, is also active against HBV but has not been approved for HBV treatment. These agents all suppress HBV replication but cannot directly eliminate cccDNA; thus, once therapy is discontinued, relapse of viremia occurs in the majority of HBeAg-positive patients who do not achieve HBeAg seroconversion and in nearly all HBeAg-negative patients.

Lamivudine

Lamivudine is the negative enantiomer of 2′,3′dideoxy-3′-thiacytidine (see Chapter 47). It is phosphorylated by host enzymes, and the incorporation of the triphosphate form into DNA results in premature chain termination, which inhibits HBV DNA synthesis. Lamivudine is administered at 100 mg/day in those without HIV infection and is well tolerated, with side effects no different than placebo. In patients coinfected with HIV, 300 mg/day is given to inhibit both HIV and HBV replication. Dose reduction is necessary for patients with renal insufficiency (creatinine clearance [CrCl] < 50 mL/min). Several large, randomized clinical trials in patients with HBeAg-positive and HBeAg-negative chronic hepatitis B demonstrate the efficacy of lamivudine (see Table 145.4).[289,290] A multinational study of Asian patients showed that HBeAg seroconversion rates increased over time from 17% to 27% at 2 years to 50% by 5 years.[291,292] An important predictor of HBeAg seroconversion at 1 year is the pretreatment aminotransferase level, ranging from 5% in patients with ALT less than two times the ULN to 64% in those with ALT greater than five times the ULN.[293] Patients with HBeAg-negative hepatitis B appear to have similar response rates,[294–296] as do patients who have previously failed IFN-α therapy.[297] Genotype, as with all the oral agents, is not a known predictor of response. Resistance to lamivudine develops when used as monotherapy limiting the durability of response; thus it is not a recommended first-line agent (see "Viral Resistance" later).

Adefovir

Adefovir dipivoxil is the oral prodrug of adefovir, a phosphonate nucleotide analogue of adenosine monophosphate (see Chapter 47). It is effective against both wild-type and lamivudine-resistant HBV[298–300]; however, it is the least potent of the oral agents (see Table 145.4) and is not a recommended first-line agent. Adefovir is administered at 10 mg daily with adjustment for renal insufficiency, and it should not be given with TDF or TAF.

In clinical trials, headache and abdominal pain are the most common side effects. Nephrotoxicity occurs in 3% of patients with compensated liver disease after 4 to 5 years of continued adefovir and in 47% of patients who underwent a liver transplant.[301,302]

Entecavir

Entecavir is a guanosine analogue that inhibits all three functions of the HBV polymerase: priming, negative-strand reverse transcription, and synthesis of the positive-strand HBV DNA (also see Chapter 47). It received US Food and Drug Administration (FDA) approval for the treatment of HBeAg-negative and HBeAg-positive chronic

TABLE 145.4 Approved Agents for Treatment of Chronic Hepatitis B With 48-Week Response

	PEG IFN-α	LAMIVUDINE	ADEFOVIR	ENTECAVIR	TELBIVUDINE	TENOFOVIR DF/AF
Route	Subcutaneous	Oral	Oral	Oral	Oral	Oral
Dose	180 μg/wk	100 mg/d[b]	10 mg/d[b]	0.5 mg/d[b] (1 mg/d if lamivudine resistant)	600 mg/d[b]	300 mg/d[b] for TDF and 25 mg/d for TAF
Duration (wk)	48	≥48	≥48	≥48	≥48	≥48
Tolerability	Fair-poor: flulike symptoms	Good	Good: follow renal function	Good	Good	Good: follow renal function and bone mineral density on TDF
HBeAg seroconversion	27%	16%–21%	12%	21%	22%	21%
Undetectable HBV DNA[c]	25%–63%	40%–73%	21%–51%	67%–90%	60%–88%	76%–93%
ALT normalization	38%	41%–75%	53%	72%	65%	74%
HBsAg loss	3%	<1%	0%	2%	<1%	3%
Viral resistance	None	15%–30%	Minimal	None[d]	6%	0%

[a]All data are for 1 year unless otherwise noted.
[b]Dose adjustment for creatinine clearance.
[c]Higher end of range for HBeAg-negative disease.
[d]None; otherwise, 7% if preexisting lamivudine resistance after 48 weeks and 50% after 5 years.
ALT, Alanine aminotransferase; *anti-HBe,* antibody to hepatitis B e antigen; *HBV,* hepatitis B virus; *HBsAg,* hepatitis B surface antigen; *PEG IFN-α,* pegylated interferon-α; *TAF,* tenofovir alafenamide; *TDF,* tenofovir disoproxil fumarate.
Modified from Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. *2009;50:661–662.*

hepatitis B in 2005. As one of the most potent anti-HBV agents, it is one of the first-line therapies for chronic hepatitis B in patients without lamivudine-resistant HBV. The recommended dose of entecavir is 0.5 mg/day for those who are treatment naïve and 1 mg/day in those with prior lamivudine experience because the activity is lower against lamivudine-resistant HBV. The dose should be adjusted for those with CrCl <50 mL/min. In a double-blind controlled trial of 709 HBeAg-positive patients, the 354 who received 48 weeks of entecavir had a decline in HBV DNA of 6.9 $\log_{10}$ copies/mL, which was superior to the 5.4 $\log_{10}$ copies/mL in the 355 who received lamivudine.[303] Furthermore, 67% in the entecavir group compared with 36% in the lamivudine group achieved HBV DNA that was <300 copies/mL, and histologic improvement occurred in 72% and 62% in the entecavir and lamivudine groups, respectively. However, HBeAg seroconversion was similar between the groups. Results after 96 weeks of treatment showed similar efficacy rates.[304] Similarly, in the HBeAg-negative patients, 70% of the 331 in the entecavir group had histologic improvement compared with 61% of the 317 in the lamivudine group. An undetectable HBV DNA was achieved in 90% in the entecavir group compared with 72% in the lamivudine group. In a study from China, after 7 years of entecavir undetectable HBV DNA was achieved by 96.8% of the HBeAg-positive patients and 100% of the HBeAg-negative patients.[305] Side effects to entecavir include headache, abdominal pain, and diarrhea.

Entecavir inhibits HIV-1 replication and can select for the lamivudine-resistant HIV mutation M184V when given as monotherapy[306]; thus HIV status should be checked before initiating therapy with entecavir. Furthermore, it should not be given to an HIV-coinfected patient without a suppressive HAART regimen.

Telbivudine

Telbivudine is an L-nucleoside analogue that is structurally related to lamivudine (see Chapter 47) and thus has overlapping resistance patterns at the rtM204 position. Telbivudine is administered at 600 mg daily and has potent activity. However, due to relatively high rates of resistance and cross-resistance with lamivudine, it is not a first-line agent. It is controversial as to whether it inhibits HIV-1 replication. The GLOBE trial, the registration trial for telbivudine, was an international study randomizing patients to telbivudine ($n = 680$) or lamivudine ($n = 687$) daily.[307] At 52 weeks the proportion with undetectable HBV DNA (<300 copies/mL) was greater in the telbivudine than the lamivudine group (60% vs. 40%, respectively). At week 104 telbivudine continued to be superior, but the proportion with undetectable HBV DNA decreased to 56%. These changes resulted from the development of resistance at rtM204I, which reached 25% at week 104. Results of the HBeAg-negative group also showed superiority of telbivudine over lamivudine and demonstrated poor durability of response due to development of resistance, which occurred in 8.6% of the telbivudine group.

Tenofovir Disoproxil Fumarate (TDF) and Tenofovir Alafenamide (TAF)

TDF was approved for treatment of hepatitis B in 2008 and is also approved for treatment of HIV-1 (see Chapters 47 and 128). TDF is potent, and resistance has not clearly been identified; thus it is a recommended first-line agent in patients with and without lamivudine-resistant HBV. TDF is dosed at 300 mg daily and needs to be adjusted for renal insufficiency. In two phase III trials comparing TDF with adefovir, viral suppression occurred more often in those receiving TDF than adefovir dipivoxil in both HBeAg-negative (93% vs. 63%, $P < .001$) and HBeAg-positive patients (76% vs. 13%, $P < .001$) after 48 weeks.[264] Of significance, more HBeAg-positive patients treated with TDF than those treated with adefovir dipivoxil had normalized ALT levels (68% vs. 54%, $P = .03$) and loss of HBsAg (3% vs. 0%, $P = .02$). TDF produced a similar HBV DNA response in patients regardless of prior lamivudine use. Continued use of TDF up to 7 years demonstrates that 99% of the HBeAg-positive and HBeAg-negative patients on treatment have HBV DNA <29 IU/mL.[308] No signature TDF-resistance mutations have been identified. The major side effects of TDF are renal insufficiency with increases in serum creatinine and decrease in bone mineral density.

TAF has equivalent anti-HBV activity to TDF but has less kidney and bone toxicity.[309,310,311] It was approved for treatment against hepatitis B in November 2016 and is given at a dose of 25 mg once daily. Two randomized, double-blinded studies compared TAF with TDF for the treatment of HBeAg-positive and HBeAg-negative chronic hepatitis B. After 48 weeks of treatment, HBV DNA of <29 IU/mL was achieved in 94% of the TAF group and in 93% of the TDF group in the HBeAg-negative study.[310] The proportions were 64% and 67% for TAF and TDF, respectively, in the HBeAg-positive study.[311] In both studies the patients receiving TAF had significantly smaller declines in bone mineral density and lower changes in glomerular filtration rate.

Other Agents and Combination Therapy

The other agent that is approved for HIV treatment but is also active against HBV is emtricitabine. Emtricitabine is similar to lamivudine, but it has a longer half-life. Mutations that confer resistance to lamivudine also confer resistance to emtricitabine (see Chapters 47 and 128).

Combination therapy for hepatitis B is attractive because it may increase potency and decrease the rates for developing resistance; however, these concepts have not been clearly demonstrated to date, so combination therapy is not generally recommended. PEG IFN-α and lamivudine for 48 weeks had greater HBV DNA decline compared with monotherapy with either one alone; however, the relapse rate in the combination group was higher, so 24 weeks after the end of treatment, there was no difference between the PEG IFN-α group alone or with lamivudine.[36,265] Another study showed that adding PEG IFN-α for 24 weeks after 24 weeks of entecavir improved HBV DNA, HBeAg, and HBsAg responses compared with continuing entecavir for 48 weeks; however, this study did not have a PEG IFN-α monotherapy arm.[312] Other studies have not shown a benefit of PEG IFN-α added after 12 weeks of entecavir compared with PEG IFN-α alone.[313,314] In a randomized, open-label study, the combination of TDF and PEG IFN-α demonstrated greater HBsAg loss (9%) compared with either TDF or PEG IFN-α alone (0% and 2.8%, respectively); however the rates were low in all groups.[315] Another randomized open-label study of HBeAg-positive patients on TDF or entecavir for more than12 months with HBV DNA <2000 IU/mL found that adding PEG IFN-α compared with continuing the nucleoside alone for 48 weeks had greater HBeAg seroconversion, but it was not statistically significantly different (18% vs. 8%, $P = .31$).[316]

Given the expansion in the number of oral agents available, it is desirable to consider combining these as is done in HIV therapy. The oral anti-HBV drugs all target the same part of the HBV life cycle; thus the situation is not entirely analogous to HIV, in which drugs target different aspects of the viral life cycle. In a randomized, open-label study combining the two most potent agents, entecavir and TDF compared with entecavir alone, there was no difference after 96 weeks of therapy between the 197 subjects receiving combination therapy and the 182 subjects receiving entecavir alone for the primary end point (<50 IU/mL) of HBV DNA (83.2% and 76.4%, respectively, $P = .088$).[317] However, when stratified by HBeAg status, the HBeAg-positive patients who received combination therapy were more likely to have a virologic response compared with the monotherapy group ($P = .046$), which was entirely attributable to those with baseline HBV DNA $>10^8$ IU/mL. There were no differences in HBeAg or HBsAg seroconversion in any groups. Thus this study suggests that combination therapy may be most beneficial in those with high HBV DNA levels, but more studies of longer duration are needed to confirm this.

Viral Resistance

The major limitation in the use of oral agents for hepatitis B is the development of resistance, which has been observed with the use of all approved oral agents except tenofovir (DF and AF), albeit at different times and frequencies. Resistance mutations differ based on the structural group of the drug, which include: L-nucleosides, D-cyclopentanes, and acyclic nucleotides. Cross-resistance occurs between agents in the same group and may decrease sensitivity between groups. The L-nucleosides group consists of lamivudine, emtricitabine, and telbivudine, with the primary resistance mutation occurring at position rt204 of the HBV polymerase, which is essential for polymerase activity, and leads to

substitution of isoleucine (I) or valine (V) or serine (S) for methionine.[291,318,319] Of the L-nucleosides, genotypic resistance develops most rapidly to lamivudine, with 14% to 32% of patients having evidence of mutations after 1 year of treatment.[289,290] Cumulative resistance increases over time, with one study demonstrating rates of 15%, 38%, 55%, 67%, and 69%, at 1, 2, 3, 4, and 5 years, respectively.[291] HBV mutants with the rtM204V/I appear to have reduced replication capacity in vitro and in vivo compared with wild-type HBV.[320] However, the development of a compensatory mutation at rtL180M restores replication capacity, at least in vitro.[320] The rates of lamivudine resistance in patients treated for HBeAg-negative chronic hepatitis B appear to be more variable (0%–27% at 1 year and 10%–56% at 2 years).[296,321]

Although the rates of resistance to telbivudine are lower than to those of lamivudine (11% vs. 26% at 2 years), resistance is high enough to preclude its widespread use.[322] Telbivudine is not active against HBV strains bearing lamivudine mutations rtL180M/rtM204V/I but remained active against the rtM204V single mutant in vitro, potentially explaining the difference in resistance profiles between telbivudine and lamivudine. Against HBV genomes with known telbivudine-resistance mutations, rtM204I and rtL80I/M204I, telbivudine, lamivudine, and entecavir lost 353- to >1000-fold activity, whereas adefovir and TDF exhibited no more than threefold to fivefold change.[323]

The second group, D-cyclopentanes, consists of entecavir, which has a high genetic barrier to resistance as it requires the presence of multiple mutations, including the rtM204V and rtL180M mutations, along with either the rtT184S/A/I/L or rtC202G/C or rtM250 mutations. In patients who are treatment naïve and do not have a preexisting rtM204V or rtL180M mutation, the rates of resistance are <1% per year with a 5-year cumulative rate of 1.2%.[324] However, in patients with the characteristic lamivudine mutations rtM204V and rtL180M, the cumulative incidence is 51% after 5 years; therefore treatment with tenofovir (DF or AF) is preferred over entecavir in patients with lamivudine-resistant HBV. Due to overlapping resistance mutations with lamivudine, lamivudine should be discontinued when entecavir is started, to theoretically decrease the risk for developing entecavir resistance.

The third group, acyclic nucleotides, consists of tenofovir (DF and AF) and adefovir dipivoxil. Resistance to adefovir dipivoxil occurs at residue rtN236T and rtA181V/T of the HBV polymerase. After 240 weeks of treatment the cumulative probability of resistance was 29% for HBeAg-negative patients. In patients with prior lamivudine-resistant mutations, cumulative incidence of adefovir dipivoxil resistance was higher—43% at 4 years.[325] In HBV cell lines expressing adefovir dipivoxil resistance mutations rtN236T and rtA181V, telbivudine remained active as shown by respective changes of 0.5-fold (rtN236T) and 1.0-fold (rtA181V and rtA194T).[323]

TDF resistance rates appear low, but precise mutations with confirmed resistance to TDF have not been characterized. Through 5 years of therapy, TDF maintains HBV DNA suppression, and there is no clear evidence of resistance.[326] The adefovir resistance mutations rtA181T/V and rtN236T confer decreased sensitivity to TDF. In one retrospective study, patients on TDF with these mutations were less likely to achieve HBV DNA <400 copies/mL than those without the mutations.[327] Because TAF is a different prodrug than TDF, the resistance patterns will be the same.

Resistance is suspected with the reappearance of HBV DNA in a patient who was previously undetectable or with a >1 $\log_{10}$ increase in HBV DNA in a patient who is compliant with therapy. Serum aminotransferases may or may not become elevated, and there are rare instances of acute exacerbations and hepatic decompensation.[328] Commercial assays are available to detect resistance mutations. If resistance occurs, current recommendations are to change therapy to the most effective agent that does not share cross-resistance. In general, the L-nucleosides (lamivudine, telbivudine, and entecavir) are not cross-resistant with the acyclic nucleotides (adefovir, tenofovir DF/AF), with tenofovir DF or AF preferred. In cases of multidrug resistance a combination of entecavir and tenofovir DF or AF is recommended.[329,330]

Given the overlapping reading frames, mutations in the HBV polymerase can lead to changes in the surface antigen, including in the immunogenic "a" determinant. One study demonstrated that a triple mutant that occurs on lamivudine monotherapy (rtV173L/rtL180M/rtM204V) leads to the surface changes sE164D/I195M. These changes in the surface antigen lead to reduced binding of anti-HBs in vitro, and thus the virus behaves as a vaccine escape mutant.[331]

Monitoring and Treatment Duration of Nucleos(t)ide Therapy

Virologic response to nucleos(t)ide analogue therapy is based on HBV DNA levels and should be evaluated every 3 months after initiating or changing therapy until the HBV DNA is undetectable and then monitored every 3 to 6 months. In addition to HBV DNA, HBeAg (in HBeAg-positive patients), and ALT should also be monitored (Table 145.5). If there is less than a 1-log IU/mL decline in the first 3 months, this is defined as primary nonresponse, which is most commonly due to noncompliance, especially if a patient is on entecavir, TDF, or TAF. If a patient with a primary nonresponse is compliant, then resistance testing is appropriate to determine a change in the treatment strategy. After 12 months of therapy a virologic response is defined as having an undetectable HBV DNA by a sensitive PCR assay. However, if after 12 months of therapy in a patient who is compliant, the HBV DNA is detectable but has declined more than 1 $\log_{10}$, then this is defined as a partial virologic response. In this case a treatment-naïve patient receiving entecavir, TDF, or TAF can continue the same therapy because there is a low rate of resistance. In a patient receiving a less potent drug, changing to entecavir, TDF, or TAF is recommended. In patients with low-level persistent viremia on entecavir, TDF, or TAF, there is little evidence that adding a second drug is beneficial. Virologic breakthrough can occur at any time and is defined as >1 log IU/mL increase in HBV DNA from the nadir value on therapy. The main causes of virologic breakthrough are noncompliance with therapy or viral resistance (see "Viral Resistance" earlier).

The duration of therapy for most individuals is lifelong, but there are some patients for whom discontinuation of therapy can be considered. For patients with HBeAg-positive chronic hepatitis B who have HBeAg seroconversion, current recommendations are to treat for 1 year after seroconversion and then consider discontinuing therapy if HBV DNA is undetectable and ALT is normal.[250] The exception

TABLE 145.5 Virologic Response to Nucleos(t)ide Treatment

RESPONSE	EVALUATION TIME (MONTHS)	CRITERIA	THERAPEUTIC STRATEGY
Primary nonresponse	3	<1 $\log_{10}$ decline	Assess compliance. If compliant, obtain resistance test and change treatment.
Partial virologic response	12	HBV DNA detectable but >1 $\log_{10}$ decline from baseline	If treatment naïve and taking entecavir or tenofovir DF/AF, then continue. If treatment naïve and taking less potent drug, change to entecavir or tenofovir DF/AF. If treatment experienced on entecavir, either change to or add tenofovir DF/AF.
Virologic response	12	HBV DNA undetectable by sensitive PCR assay	Continue treatment and monitor every 3–6 mo.
Virologic breakthrough	Any time	>1 $\log_{10}$ increase in serum HBV DNA level from on therapy nadir	Obtain resistance test and change therapy (see "Viral Resistance" in text).

AF, Alafenamide; *DF,* disoproxil fumarate; *DNA,* deoxyribonucleic acid; *HBV,* hepatitis B virus; *PCR,* polymerase chain reaction.

to this is that patients with cirrhosis should not be considered for discontinuation of therapy. Once HBeAg seroconversion has occurred, the rate of durable response after discontinuation ranges from 30% to 80%.[332] However, due to the possibility of relapse, some recommend continuing therapy until HBsAg seroconversion, which is infrequent. In one study recurrent viremia occurred in 90% of the 39 patients who stopped therapy a median of 12 months after seroconversion, compared with 0% in those who continued therapy, so monitoring every 3 months with serum aminotransferases and HBV DNA is important after discontinuation of therapy.[250,333] There is some evidence that quantitative HBsAg may be useful to predict who will relapse after therapy discontinuation.[334] If a patient does not have HBeAg seroconversion but has suppressed HBV DNA replication, then therapy should be continued indefinitely because there is evidence of progressive enhancement of the HBeAg seroconversion rate with longer duration of treatment, and patients may continue to derive histologic benefit.[267] In HBeAg-negative patients therapy should be continued indefinitely because relapse occurs in the majority of cases except in rare instances of HBsAg seroconversion. However, the European guidelines suggest that, in selected noncirrhotic HBeAg negative patients with undetectable HBV DNA for at least 3 years, discontinuation of therapy could be considered with close monitoring.[65] In support of this, one study of HBeAg-negative patients with undetectable HBV DNA for ≥3.5 years found that 13 of 21 people who discontinued TDF remained off therapy through 144 weeks.[335] In both HBeAg-negative and HBeAg-positive disease, therapy discontinuation can be considered 6 to 12 months after HBsAg seroconversion, which only occurred in 8% after 6 years of TDF.

MANAGEMENT IN SPECIAL POPULATIONS

Liver Transplantation

Recurrent hepatitis B after liver transplantation can occur in patients with a history of acute or chronic hepatitis B before transplantation or in HBsAg-negative recipients of anti-HBc–positive donors. Historically, rates of recurrence of HBV infection were high (90%), and the consequences of reinfection were devastating, such that HBV was considered a relative contraindication to liver transplantation. HBIG was introduced in the early 1980s and nucleosides in the early 1990s, with progressive reduction in likelihood of HBV recurrence with each intervention. Using HBIG alone, recurrence rates ranged from 20% to 25%, depending on the schedule and trough anti-HBs titer. The addition of oral nucleos(t)ide therapy (lamivudine) to HBIG was shown to further decrease recurrence rates to as low as 10% and improve mortality.[336,337] Similar to nontransplant patients, however, treatment with lamivudine alone resulted in the emergence of lamivudine resistance and severe and even fatal posttransplantation disease.[338] Tenofovir (DF or AF) or entecavir are now the preferred nucleos(t)ide analogues and are >95% effective in protecting the grafts from recurrent disease. Both have been shown to suppress lamivudine-resistant variants[298] and even resolution of graft failure in those patients who have them.[339] In a recent study evaluating long-term outcomes in 265 chronic HBV liver transplant recipients treated with entecavir without HBIG, followed for a median of 59 months, 92% were negative for HBsAg at 8 years, and 100% had undetectable HBV DNA. Eighty-five percent of the patients were alive at 9 years.[340] In a randomized controlled trial comparing TDF and emtricitabine with and without HBIG in orthotopic liver transplant recipients, followed for a median of 3.4 years, no recurrences of HBV occurred in either arm, suggesting therapy with TDF and emtricitabine without HBIG is effective at reducing HBV recurrence.[341] Although an increasing number of transplant centers are using nucleos(t)ide analogues alone for post-transplant prophylaxis, HBIG continues to be recommended in certain high-risk patients, including individuals with high HBV DNA levels, known antiviral resistance, higher risk for recurrence of HCC, HIV coinfection, and HDV coinfection.[342] Finally, although TDF is most studied in posttransplant patients, TAF is a better alternative due to its safer renal profile. In summary, with currently available combination therapy, survival is excellent in patients undergoing liver transplantation for chronic hepatitis B, even in those with active viral replication pretransplantation.

Vaccination after transplantation has variable success; in one study, active immunization after liver transplantation induced anti-HBs titers (>10 UI/mL) in 82% of liver transplant candidates with inactive HBV disease[343]; however, in a later study of liver transplant recipients with cirrhosis who received conventional HBV vaccine after transplantation, only 18% achieved protective anti-HBs titers.[344] Other reports of conventional vaccine failure after liver transplantation have been reported.[345,346] The use of repeated immunization with recombinant HBV vaccine emulsified in novel adjuvants in patients who were HBV DNA negative after orthotopic liver transplantation resulted in anti-HBs levels ranging from 721 to 83,121 IU/L, depending on the concentration, in 80%, which remained stable after discontinuation of HBIG.[346] However, in a more recent study of eight liver transplant recipients with similar characteristics but in whom HBIG was stopped, the use of an adjuvant containing HBV vaccine resulted in development of anti-HBs in only one patient.[347]

Human Immunodeficiency Virus

The efficacy of the nucleos(t)ide agents does not seem to be compromised by coinfection with HIV.[348] However, with lamivudine monotherapy, drug resistance develops more rapidly than in HBV monoinfection.[349] Lamivudine-resistant HBV is common in HIV coinfection because it has been a backbone agent for HIV therapy for years. Tenofovir (DF and AF) has potent activity against both HIV and HBV[350] and is also effective against lamivudine-resistant HBV.[351–353] The combination of TDF or TAF and emtricitabine (or TDF and lamivudine) is therefore now recommended in the treatment of chronic HBV in HIV-infected individuals.[354] TAF is less likely to adversely impact renal function and bone mineral density without compromising efficacy and thus is preferred over TDF when CrCl > 30 mL/min. The three approved fixed-dose TAF-containing regimens include elvitegravir/cobicistat/emtricitabine/TAF; emtricitabine/rilpivirine/TAF; and emtricitabine/TAF. Current Department of Health and Human Services (DHHS) HIV treatment guidelines recommend initiation of ART in all HIV-infected patients, including those with HIV/HBV coinfection.[354] If TDF or TAF cannot be safely used, entecavir should be used with a fully suppressive ART regimen because entecavir can also inhibit HIV replication and select for the HIV drug-resistance mutation M184V.[306] A higher dose of entecavir (1 mg) should be used in patients who have already received lamivudine or have known lamivudine resistance. However, due to the relatively high rate of resistance of entecavir in those with lamivudine resistance (see "Viral Resistance" earlier), HBV DNA should also be monitored frequently.[354] If HBV needs treatment and a fully active anti-HIV regimen cannot be given, then drugs that are active only against HBV and do not lead to HIV drug resistance, such as adefovir dipivoxil or PEG IFN, are recommended. Telbivudine is not recommended, given its intermediate rate of resistance in HBV monoinfection and unknown rates in HIV coinfection. Treatment is indefinite unless HBsAg seroconversion occurs.

Recipients of Immunosuppressive Therapies

A number of cytotoxic chemotherapeutic agents, immunomodulators, and immunosuppressants have been associated with reactivation of HBV. Agents that have been most commonly associated are prednisone, anthracyclines, and anti-CD20 blockers, specifically rituximab or ofatumumab as part of chemotherapy.[355] The frequency of reactivation in patients undergoing cytotoxic chemotherapy ranges from 14% to 72% in different studies and case series, depending on serologic status of the patient and potency of immunosuppressants. Patients at risk for reactivation include those with resolved infection (HBsAg negative, anti-HBs, and anti-HBc positive), those with inactive chronic hepatitis B (HBsAg positive, HBV DNA undetectable), and those with untreated chronic active hepatitis B (HBsAg positive, HBV DNA detectable). Tumor necrosis factor-α (TNF-α) inhibitors, including infliximab, etanercept, and adalimumab and low-dose steroids, have a low-to-moderate risk of reactivation. Very few cases of reactivation have been reported with the use of immunosuppressants, including azathioprine and methotrexate.[355] Anthracyclines carry a moderate risk for HBV reactivation in HBsAg-negative, anti-HBc–positive patients and a high

risk in HBsAg-positive patients. The risk of reactivation of HBV associated with rituximab, a monoclonal antibody directed against B-cell marker CD20 and increasingly used in standard chemotherapy regimens for many rheumatologic disorders, carries the highest risk of reactivation in patients who are anti-HBc positive, with or without anti-HBs. In a recent meta-analysis of case series and reviews of HBV reactivation in lymphoproliferative disorders associated with rituximab, there was a more-than-fivefold higher rate of HBV reactivation in patients with a history of prior HBV exposure (anti-HBc positive, with or without anti-HBs) who received rituximab-based therapy, compared with those who did not.[356] In a study of 46 HBsAg-negative/anti-HBc–positive patients (the majority of whom were anti-HBs positive), 25% of patients receiving rituximab and cyclophosphamide-hydroxydaunorubicin-Oncovin (vincristine)-prednisone (CHOP) for diffuse large B-cell lymphoma reactivated HBV (became HBsAg positive) compared with 0% who received CHOP alone.[357] In 2013 the FDA issued a black box warning concerning the risk of HBV reactivation in patients receiving anti-CD20 monoclonal antibodies after more than 100 cases of fatal HBV-related liver failure associated with rituximab or ofatumumab were reported in postmarketing data from the FDA adverse-event report system between 1997–2012.[357a]

Prophylaxis is recommended in HBsAg-positive patients at highest risk of reactivation, if untreated, especially if they are taking B-cell–depleting drugs, anthracycline derivatives, or high-dose steroids or receiving bone marrow transplants.[358] Anti-HBc–positive patients who are HBsAg positive or negative and at moderate risk of reactivation, that is, patients on TNF-α inhibitors, low-dose steroids, and anthracycline inhibitors (if HBsAg negative) can be closely monitored or receive prophylaxsis.[355] Although lamivudine is the most widely studied antiviral agent (associated with reductions in risk of HBV reactivation of 79%–100%), tenofovir (DF or AF) and entecavir are now preferred, especially when the duration of prophylaxis is expected to be longer than 1 year. Entecavir and TDF have higher antiviral potency and lower rate of resistance with long-term use. In a randomized trial in patients with diffuse large B-cell lymphoma receiving chemotherapy with rituximab-CHOP (R-CHOP), entecavir was found to be superior to lamivudine for the prevention of HBV reactivation and HBV-related hepatitis in those with prechemotherapy HBV DNA ≥2000 IU/mL. TDF has also shown to be effective in reducing HBV reactivation in anti-HBc–positive patients with hematologic malignancies treated with rituximab.[359] The optimal duration of prophylactic therapy is unknown; current recommendations are to start preferred antiviral drugs with immunosuppressive medication and continue 6 to 12 months after the last dose of treatment. For patients receiving B-cell–depletion agents, therapy should be continue 12 to 18 months after the last dose, as reactivations as late as 17 months after completion of therapy have been reported.[250,360,361] Among patients with higher levels of baseline HBV DNA (>2000 IU/mL), the American Association for the Study of Liver Disease (AASLD) recommends continuing antiviral therapy until they reach treatment end points for HBV infection.[250] Among bone marrow transplantation recipients, the risk of reactivation of HBV in HBsAg-positive patients is up to 54%.[358] The use of preemptive antivirals therefore, in these situations, is also recommended. Of interest, a donor bone marrow with natural immunity (anti-HBs and anti–HBc positive) may result in clearance of HBsAg.[362]

Treatment of Hepatitis B Virus During Pregnancy

All women presenting for prenatal care should be routinely tested for HBsAg early in their pregnancy. There are at least three indications for treatment of HBV during pregnancy: (1) to treat active liver disease in situations when therapy cannot be delayed, (2) the continuation of HBV treatment in a mother who becomes pregnant while on HBV therapy, and (3) to prevent transmission of HBV from mother to child in mothers with high circulating viremia. All treatment decisions need to take into consideration the risks and benefits for mother and fetus (potential exposure to teratogenic drugs). TDF (category B) and lamivudine (category C) have been studied most extensively in pregnancy. In the Antiretroviral Pregnancy Registry and the Development of Antiretroviral Therapy study (DART), birth defects among women exposed to HBV therapy (mostly TDF and lamivudine) were similar to that in the general population in the first and second trimesters and to other classes of antiretrovirals.[363] Similarly, to date no adverse outcomes have been reported with telbivudine (category B).[364] A recent systematic review and meta-analysis of antiviral therapy in chronic HBV found no evidence of fetal adverse effects or congenital defects associated with TDF.[364] There are minimal data on the safety of adefovir or entecavir or TAF (category C) in pregnancy; thus they should be avoided in pregnancy until more clinical data are available. Interferon is contraindicated in pregnancy. Based on data demonstrating a decrease in perinatal transmission, current guidelines recommend treatment of pregnant women in the third trimester when HBV DNA > 200,000 IU/mL (see also "Prevention of Perinatal Transmission" later). The guidelines also recommend that pregnant women in the immunoactive phase are managed the same as nonpregnant women, with the caveat that only drugs tested in pregnant women should be used.[250]

OTHER MANAGEMENT ISSUES IN CHRONIC HEPATITIS B

Patients with chronic hepatitis B should be counseled about disease-modifying factors, as well as means to prevent spread of HBV to other persons. For example, patients should be counseled about the means of spread of delta hepatitis and hepatitis C to avoid superinfection with these viruses. Patients should also be counseled to consume minimal, if any alcohol, in the absence of data regarding safe levels of consumption and because consumption of large amounts of alcohol is clearly a risk factor for more rapid progression to cirrhosis. Other major issues in the management of patients with chronic hepatitis B include prevention of hepatitis A, prevention of spread of HBV, and surveillance for HCC.

Hepatitis A Vaccination

The official recommendation of the Advisory Committee for Immunization Practices (ACIP) in the United States is that all persons with chronic liver disease be vaccinated against hepatitis A virus (HAV).[365] The data supporting this recommendation are not strong[366] because at least one study revealed that the risk of fulminant HAV is significantly increased only in patients with underlying hepatitis C and not hepatitis B.[367] However, the guidelines of the AASLD call for immunization of all patients with chronic HBV against HAV.

Screening and Vaccination of Contacts

Sexual and household contacts of persons with HBV are at increased risk of infection. All sexual partners and household contacts should be tested for HBV and vaccinated if seronegative. Until the immunization series is complete, sexual partners should use barrier methods. Both patients and contacts should be counseled regarding the modes of transmission and advised on methods to prevent household transmission, including avoiding sharing of items that might be contaminated with small amounts of blood, such as toothbrushes, and the need to cover open wounds. Pregnant women or women who want to become pregnant and are infected with HBV should also be counseled on the risk of transmission to the newborn and the method to prevent such transmission. Postexposure prophylaxis with HBIG and hepatitis B vaccine should be given to infants born to HBsAg-positive mothers, unvaccinated infants whose mothers or primary caregivers have acute hepatitis B, sexual contacts of persons with hepatitis B, and health care workers after occupational exposure to HBsAg-positive blood depending on their vaccination and vaccine response status. Household and sexual contacts of persons with chronic HBV infection should be vaccinated.

Recommendations for the health care worker infected with HBV vary from country to country. Although there is general agreement that individuals with HBeAg or HBV DNA >10,000 IU/mL pose the greatest risk of transmission,[368] there have been documented cases of transmission from health care workers in the absence of HBeAg and during "low-risk" procedures.[369,370] Updated Centers for Disease Control and Prevention (CDC) guidelines published in 2012 recommend that most chronically HBV-infected providers and students who adopt standard infection control precautions do not require curtailing their practices. Previous recommendations were updated to also include the following: (1) prenotification of a health care provider's or student's HBV status is no longer required; (2) the use of HBV DNA serum levels rather than

HBeAg status is recommended to monitor infectivity; and (3) for those health care professionals requiring oversight (i.e., health care worker performing highly exposure-prone procedures), there are specific suggestions for composition of expert review panels and threshold value of serum HBV DNA considered "safe" for practice (<1000 IU/mL).[371]

Surveillance for Hepatocellular Carcinoma

In multiple longitudinal studies, carriers of HBsAg have been shown to be at increased risk of developing HCC.[372] The risk is higher among males and those who are older, have a family history of HCC, consume alcohol regularly, have elevated serum ALT levels, have positive HBeAg status, have hepatitis B DNA >10^4 copies/mL (≈2000 IU/mL), have genotype C virus, have cirrhosis, and have a longer duration of infection.[177,373,374] The goal of HCC screening is to detect small, surgically resectable tumors because the prognosis for more advanced lesions is poor. Current recommendations are that HBsAg-positive persons at high risk for developing HCC should be periodically screened; these include Asian males older than 40 years, Asian females older than 50 years, African/North American blacks older than 20 years, those with a family history of HCC, those with cirrhosis, and persons with ALT elevations or HBV DNA >2000 IU/mL, or both.[250] In a single, randomized, controlled trial conducted in China comparing surveillance using ultrasonography and α-fetoprotein levels with no surveillance, the incidence of HCC-related mortality was reduced by 37%.[375] However, there are no randomized trials of HCC surveillance in US populations, and several studies show that α-fetoprotein determination lacks adequate sensitivity and specificity. Therefore the current recommendation for HCC screening is with ultrasonography alone every 6 months.[375] The role of other imaging technologies, such as computed tomography, in screening have not been adequately assessed. Although the risk of HBV is decreased in patients achieving viral suppression, it is not eliminated, especially in the setting of cirrhosis. A study following Chinese patients with chronic hepatitis B on adefovir for 10 years found that the incidence of HCC in cirrhotic patients was 43.1% compared with 7.1% in noncirrhotics.[376] Furthermore, cirrhotic patients who achieved virologic suppression had a lower incidence of HCC than those who did not (27.8% vs. 62.2% 10-year incidence), and the same pattern was seen in noncirrhotic patients (4.0% vs. 13.4%). Thus, even with virologic suppression, HCC occurs and surveillance every 6 months is recommended.

PREVENTION OF HEPATITIS B VIRUS INFECTION

The HBV vaccine is effective in preventing HBV and its complications. It has been shown in multiple studies to be safe, efficacious, and immunogenic, with seroconversion rates of >90% in healthy adults and seroprotection rates in infants born to HBV-infected mothers of 88.5% to 95.8%.[377–379] Successful vaccination prevents HBV infection and therefore reduces HCC-related mortality.[380] In Taiwan, which was an early adopter of universal HBV vaccination in children, the average annual incidence of HCC in 6- to 14-year-olds declined from 0.70 per 100,000 children between 1981 and 1986, before widespread vaccination, to 0.36 between 1990 and 1994, after initiation of widespread vaccination ($P < .01$).[381] The corresponding rates of mortality from HCC also decreased.

Active Immunization

Vaccines against hepatitis B have been available since 1982. The first vaccines were plasma derived and have now been completely replaced by recombinant vaccines. The vaccines contain small HBsAg as the major component produced in transfected yeast cells. Hepatitis B vaccines are available in monovalent forms for use in adults and for birth doses (Engerix-B [GSK; Philadelphia, PA] and Recombivax HB [Merck; Kenilworth, NJ]) or in combination with other vaccines for infant vaccination, including diphtheria-tetanus-pertussis (DTP), *Haemophilus influenzae* type b (Hib), and inactivated polio vaccine (IPV) (Pediarix; GSK). Pediarix can only be administered before age 6 weeks through 6 years of age. A combination vaccine (TWINRIX R; GSK), which expresses both HBsAg and hepatitis A, is also available and is approved for use in persons ≥18 years of age in the United States and Europe (see Chapter 316). Despite high efficacy of recombinant vaccines, certain populations remain at risk of suboptimal or nonresponse to these vaccines (see "Efficacy"). Recently, the first and only two-dose hepatitis B vaccine, Heplisav-B, was approved for use in adults 18 years of age and older. This novel vaccine uses HBsAg adjuvanted with immunostimulatory phosphorothioate oligodeoxyribonucleotide sequences that stimulate the innate immune system through Toll-like receptor 9 (TLR-9). The approval of Heplisav-B was based on data from three phase III noninferiority trials of nearly 10,000 adult participants who received the vaccine.[382,383] Results from the three trials have demonstrated high levels of protection early after administration with the largest trial, which included 6665 participants, demonstrating a statistically significantly higher rate of protection of 95% compared with 81% for Engerix-B. In a subgroup analysis of 961 participants with type 2 diabetes, Heplisav-B demonstrated a statistically significantly higher rate of protection of 90% compared with 65% for Engerix-B.[384] Additional subgroup analyses revealed significantly higher rates of protection among smokers, persons with body mass index ≥30 kg/m^2, and older age groups who are typically at risk of suboptimal responses with recombinant vaccines. Adults younger than 40 years had a similar response.[385] Heplisav-B has not been studied in persons infected with HIV.

Indications for Vaccination

All persons at high risk of acquiring HBV (see "Routes of Transmission" earlier) should be offered vaccination if nonimmune. Recent best-practice advice from the American College of Physicians and CDC recommends the following at-risk patient groups should be vaccinated: (1) all unvaccinated adults (including pregnant women) at risk for infection due to sexual, percutaneous, or mucosal exposure; (2) health care and public safety workers at risk for blood exposure; (3) adults with chronic liver disease and end-stage renal disease (including hemodialysis patients); (4) adults with HIV infection; (5) travelers to HBV-endemic regions; and (6) any adults seeking protection from HBV infection.[386,387] In 2011 the ACIP updated their guidelines to recommend vaccination in all adults age 19 to 59 years with diabetes. Universal vaccination of all infants has been recommended in the United States since 1991, and has since been adopted by many other countries and incorporated into routine childhood immunization programs. As of 2015, 185 (95%) of World Health Organization (WHO) member states vaccinated infants against hepatitis B as part of their vaccination schedules; global coverage with three doses of HBV vaccine during infancy reached 84%. Since the introduction of the HBV vaccine (ranging from the 1980s–2000s, varying by country) and 2015, the prevalence of HBV infection among children younger than 5 years has fallen from 4.7% to 1.3%[388] In the United States in 2014, 92% of infants had been fully vaccinated with three doses of HBV vaccine.[389] In October 2016 ACIP recommended that all US-born infants who weigh at least 2000 g receive a dose of HepB vaccine before 24 hours of age.[390]

Dose Regimen

Two single-antigen hepatitis B vaccines are currently licensed in the United States: Engerix-B and Recombivax HB. Engerix-B is formulated to contain 20 μg HBsAg/mL, and Recombivax HB contains 10 μg HBsAg/mL (Table 145.6). The recommendation for adults is to administer 1 mL of either Engerix-B or Recombivax HB in three doses at months 0, 1 to 2, and then at 6 to 12 months. In infants, three doses of 0.5 mL of vaccine are required to complete the course. For adolescents (11–19 years of age), three doses of 0.5 mL of Recombivax HB or 1 mL of Engerix-B is recommended.[391] Either vaccine can be interchanged during the series of injections. An optional two-dose regimen of 1 mL Recombivax HB has also been approved for adolescents age 11 to 15 years, with a second dose given 4 to 6 months after the first dose.[392] The recently approved Heplisav-B is administered in two doses over 1 month at 0 and 4 weeks, although there are no formal guidelines currently regarding who should receive this vaccine over the other two. However, since safety in pregnancy has not been assessed, pregnant women should receive either Engerix-B or Recombivax HB. Vaccines should be administered intramuscularly because deposition of the vaccine into adipose tissue results in a lower seroconversion rate.[393] Adverse reactions are uncommon, and most consist of soreness at the

TABLE 145.6 Doses and Schedules of Licensed Hepatitis B Vaccines

HEPATITIS B VACCINES	AGE	DOSE	VOLUME	SCHEDULE
Engerix-B	<20 yr	10 µg	10 µg/0.5 mL	Infants[b]: 1st dose within 24 h of birth, 1–4, 6–18 mo Older children: 0, 1–2, 4 mo
	>20 yr	20 µg	20 µg/1 mL	0, 1, 6 mo
	Diabetes 19–59 yr[c]	20 µg	20 µg/1 mL	0, 1, 6 mo
	Dialysis and other immunocompromised	40 µg	2–20 µg/1 mL doses	0, 1, 2, 6 mo
Recombivax HB	<20 yr	5 µg	5 µg/0.5 mL	Infants[b]: 1st dose within 24 h of birth, 1–4, 6–18 mo Older children: 0, 1–2, 4 mo
	11–15 yr	10 µg	10 µg/1 mL	0, 4–6 mo
	>20 yr	10 µg	10 µg/1 mL	0, 1, 6 mo
	Diabetes 19–59 yr[c]	10 µg	10 µg/1 mL	0, 1, 6 mo
	Dialysis and other immunocompromised	40 µg[d]	40 µg/1 mL	0, 1, 6 mo
Heplisav-B[e]	>18 yr	20 µg	20 µg/0.5 mL doses	0, 1 mo
COMBINATION VACCINES	**AGE[f]**	**ANTIGEN**	**VOLUME**	**SCHEDULE**
Pediarix	6 wk–6 yr	Engerix-B, Infanrix (DTaP), and IPV	0.5 mL	For newborns, give monovalent hepatitis B vaccine within 24 h of birth, then give Pediarix at 2, 4, 6 mo
Twinrix	>18 yr	Havrix (HAV) and Engerix-B (20 µg)	1 mL	0, 1, 6 mo

[a]All vaccines should be administered intramuscularly in the deltoid. https://www.cdc.gov/hepatitis/hbv/hbvfaq.htm#vaccFAQ.
[b]Infants born to hepatitis B surface antigen (HBsAg)-positive mothers should have hepatitis B immune globulin (HBIG) within 12 h of delivery, along with vaccine at a separate site. If mother's HBsAg status is unknown, administer vaccine within 12 h and test mother. If mother is HBsAg positive, administer HBIG within 1 wk.
[c]Hepatitis B vaccination may be administered at the discretion of the treating clinician to unvaccinated adults with diabetes mellitus who are age ≥60 yr (see http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6050a4.htm).
[d]Special formulation.
[e]Safety and effectiveness of Heplisav-B have not been established in adults on hemodialysis.
[f]Birth dose should be monovalent vaccine only; subsequent doses can be combination.
DTaP, Diphtheria, tetanus, acellular pertussis (vaccine); *IPV,* inactivated polio vaccine.

injection site. Low-grade fever, malaise, headache, and myalgias are seen in <1% of vaccinees. The vaccine can be administered during pregnancy.

Efficacy

A protective level of immune response after vaccination is defined as a titer of anti-HBs of >10 IU/L. Although this was somewhat arbitrary, clinical studies suggest that a decrease in titer below this level is associated with a risk of infection. Both Recombivax HB and Engerix-B administered over three doses, as described previously, result in a protective antibody response in >95% of adults, infants, and children. In persons receiving the newly approved Heplisav-B, 95% of patients exhibited a seroprotective response 8 weeks after the second dose of vaccine.[382] Because the response rate in healthy individuals is so high, routine postvaccination testing in persons receiving Recombivax HB and Engerix-B is not recommended, except among immunocompetent individuals who are at high risk of repeated exposure to HBV, such as health care workers, IV drug users, chronic hemodialysis patients, and individuals who are at risk for recurrent exposure to HBV (e.g., sexual partners of carriers, MSM). Testing should be performed 1 to 2 months after the vaccine series in these individuals. For these individuals who have no or inadequate anti-HBs titers after a primary series, the recommendation is to administer one or more doses of vaccine. After one to two doses, up to 25% of previous nonresponders or hyporesponders may have adequate titers. Almost all healthy adults who do not respond to a primary three-dose series with anti-HBs concentrations of >10 IU/L will respond to a three-dose revaccination series.[394] Postvaccination testing is also recommended in infants born to HBsAg-positive mothers, which should be performed at age 9 to 12 months to avoid detection of anti-HBs from HBIG administered during infancy.[395] Vaccination response is decreased in certain populations, including those older than 40 years; those who are obese, have particular genetic polymorphisms, and have a lack of sleep; and those who smoke, have HIV or any form of immunosuppression, diabetes mellitus, renal failure, chronic liver disease, improper administrations (e.g., administration into the buttock or subcutaneous injection), and freezing of the vaccine.[396,397]

Efficacy in Patients With Human Immunodeficiency Virus Infection

In patients with HIV response rates vary between 18% and 71%.[398–400] Although a strict correlation between response and immune status is not always found, response is better with CD4 T-cell counts >500 cells/mL.[401,402] US guidelines do not recommend delaying HBV vaccination in patients with HIV infection. HIV-infected persons who are nonresponders to HBV vaccine are not protected from infection and have similar rates of HBV infection as unvaccinated individuals with HIV, unlike immunocompetent individuals who may have some level of protection from the vaccine, even if anti-HBs titers fall below protective levels.[403] This is thought to be due to decreased numbers of antigen-specific memory B cells in HIV-infected individuals. A number of vaccination strategies to boost immunity in HIV-infected individuals have been examined, including accelerated schedules (four vaccine doses at 0, 1, 2, and 6 months),[404] intradermal administration, and rescue vaccination after primary vaccination, with variable success. Another strategy is use of adjuvants as stimulators of immunogenicity of the vaccine, although studies show mixed results.[405–407] Currently DHHS recommends the standard three-dose HBV vaccination, although other vaccines strategies are recommended by some experts, including the use of double-dose vaccine at 0, 1, and 6 months, with or without additional doses.[408]

Efficacy in End-Stage Renal Disease

In patients on chronic hemodialysis the response rate to recombinant vaccines is 50% to 60%.[409] In addition, anti-HBs levels decline more rapidly compared with healthy individuals.[410] Intradermal injection has been shown to be associated with higher seroconversion rates than intramuscular administration.[411,412] The CDC recommends all end-stage renal disease patients receive double doses of conventional recombinant HBV vaccine, preferably before the onset of dialysis; administration of a fourth vaccine dose may be necessary to increase the seroprotection rate (see Table 145.6). Testing 1 to 2 months after vaccination is recommended for hemodialysis patients to determine their response to the vaccine.[413] They should also undergo yearly anti-HBs

testing and receive a single booster dose of vaccine if the titer falls below 10 IU/L.

Durability of Response

There is excellent durability of response after a successful primary HBV vaccine series with Recombivax HB and Engerix-B. No long-term durability data are available from Heplisav-B. In a study from Alaska, one of the longest post-HBV vaccine cohorts, 94% of Native Alaskan children and adults who had received plasma-derived HBV vaccine at a minimum of 6 months of age in the early 1980s had evidence of immunity at 22 years of follow-up, even though only 60% of the vaccinated cohort retained detectable levels of anti-HBs. Complete protection was conferred for up to 22 years in those immunized as children and adults, and for up to 15 years in those immunized as infants.[414] A recently published study extended follow-up to 30 years and indicated that >90% of vaccines had evidence of protective immunity, based on anti-HBs levels ≥10 IU/L or responses to booster doses of vaccine.[415] A companion study indicated that evidence of T-cell immunity to HBV was present even if anti-HBs levels were not detected.[416,417] Long-term data from Taiwan, where 20% of the population is infected with chronic hepatitis B, indicate that the vaccine provides long-term protection up to 20 years in adults.[418] Among HIV-infected individuals the durability of a response to HBV vaccination is significantly associated with higher anti-HBs titers after primary vaccination, with anti-HBs responses >100 IU/L associated with the most prolonged seroprotection.[419] Breakthrough infections have been reported in successfully vaccinated individuals who were subsequently exposed to HBV after a full vaccine series; however, the number of such cases is few, and clinically significant infections are rare.

Postexposure Immunoprophylaxis

Postexposure prophylaxis with HBIG and vaccine is recommended for all nonimmune individuals who have percutaneous, sexual contact, ocular, or mucous membrane exposure to blood, including human bites that penetrate the skin (see Chapter 316), where the source is known to be at high risk of being HBsAg positive. The first dose of 0.06 mL/kg should be administered as soon as possible, preferably within 12 hours, although there is a window period of up to 24 hours.[420] The first vaccine dose should be given at the same time, although in a different site, followed by the remainder of the series. For individuals who are vaccinated but who do not have documentation of adequate titers of anti-HBs, recommendations are to administer both HBIG and a vaccine booster dose pending documentation of adequate anti-HBs. Individuals who have failed to respond to a vaccine series require two doses of HBIG 1 month apart.

Prevention of Perinatal Transmission

In women with chronic HBV infection, the risk of transmission of HBV infection to their child without prophylaxis is high and varies according to HBeAg status of mothers from 12% for HBeAg-negative/anti-HBe–positive mothers, to 25% for HBeAg-negative/anti-HBe–negative mothers, to 70% to 90% for HBeAg-positive mothers.[421–423] Maternal serum HBV DNA levels also correlate with the risk of transmission.[424] It is recommended that all neonates born to mothers with chronic hepatitis B receive HBIG 0.5 mL IM at birth and hepatitis B vaccine within 12 hours of birth, followed by vaccine dose at 1 and 6 months, which together are 85% to 95% effective in preventing perinatal HBV infection.[422,423] In a large systematic review and meta-analysis the addition of HBIG decreased risk of transmission from mother to infant compared with HBV vaccine alone[425]; however, it may not be necessary in HBsAg-positive/HBeAg-negative mothers.[426,427] Administration of a "birth-dose" HBV vaccine (within 12 hours of birth), compared with administration after 12 hours of birth, is associated with further reductions in HBV transmission, although birth-dose coverage remains low in many developing countries (39% in 2016).[428] As of 2016, 97 WHO member states have introduced the hepatitis B birth-dose vaccine.[429] Despite the relative success of immunoprophylaxis, a significant proportion of infants remain at risk for HBV transmission. Ten percent to 30% of infants develop HBV infection when mothers have high levels of viremia (defined as HBV DNA $>2 \times 5 \log_{10}$ IU/mL)[430,431,432]

Updated 2016 AASLD HBV treatment guidelines now recommend antiviral therapy beginning in the third trimester, to reduce the risk of perinatal transmission of hepatitis B in HBsAg-positive pregnant women with an HBV DNA level >200,000 IU/mL.[250] TDF remains the first-line recommended therapy based on its safety and efficacy, although telbivudine and lamivudine are also recommended. In a recent randomized, placebo-controlled trial, Pan and colleagues[433] evaluated 200 HBeAg-positive mothers with HBV DNA levels $>2 \times 5 \log_{10}$ IU/ mL and compared TDF therapy (300 mg daily) from 30 to 32 weeks of gestation to 4 weeks postpartum versus no drugs (control subjects). All infants received standard immunoprophylaxis at birth. A reduction in mother-to-child transmission for TDF compared with control subjects was found (0% vs. 7%; $P = .01$).[433] Other studies with lamivudine and telbivudine have shown similar effects.[434–436] In a recent systematic review of safety studies with TDF, many found no difference in fetal or maternal adverse effects.[436] Resistant variants have been shown in up to 19% of women receiving lamivudine in prior studies,[437] and these resistant variants rarely occur in women receiving telbivudine.[438] More data are needed on the efficacy and safety of entecavir and TAF in pregnancy before they can be recommended. Additional information is also needed on optimal timing of antiviral therapy during pregnancy, the optimal HBV DNA thresholds to treat patients with antivirals, and resistance patterns in infants with nucleos(t)ide therapy. There is no evidence that cesarean section prevents maternal-infant transmission, and thus routine cesarean section is not recommended.[439] Neither breastfeeding nor amniocentesis appears to increase the risk of transmission.[440]

Hepatitis B Surface Antigen Escape Mutants

HBs mutants have been described in infants infected with HBV after passive-active vaccination,[441] in vaccinated adults, in liver transplant recipients who have received prolonged courses of HBIG to prevent recurrence of HBV in the allograft,[442,443] and in immunocompromised patients with resolved HBV infection receiving dasatanib.[444,445] The most common mutations are the glycine-to-arginine substitution at codon 145 (G145R) and the sD144A in the "a" determinant sequence of HBsAg, which decrease binding of HBsAg to anti-HBs. The prevalence of these escape mutants is increasing over time, but the clinical and epidemiologic importance and the impact on current vaccination strategies are unclear. Selection of these HBs antigen mutants appears to be a rare event, and their prevalence even in HBV endemic areas is low.[446,447] In a chimpanzee model of infection, the current vaccines appear to protect against the spread of HBs mutants.[448]

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146 Hepatitis Delta Virus

Chloe Lynne Thio and Claudia Hawkins

SHORT VIEW SUMMARY

Definition
- Hepatitis delta virus (HDV) occurs as a coinfection or superinfection with HBV, establishing a chronic infection in hepatocytes.
- About 5% of people with chronic hepatitis B globally have evidence of exposure to HDV.
- HDV prevalence generally mirrors hepatitis B virus (HBV) prevalence, although there are geographic differences.
- HDV is primarily transmitted via the parenteral route, but there is some sexual transmission. Perinatal transmission is uncommon.

Microbiology
- HDV is a small, defective RNA virus that relies on host cell machinery for replication.
- HDV requires the envelope of HBV for viral assembly and transmission.

Clinical Presentation and Diagnosis
- Clinical presentation of HDV is variable, but both chronic and superinfection can be present as acute severe hepatitis.
- Superinfection of HDV in individuals with chronic hepatitis B results in chronic infection in greater than 90% of cases. The risk of cirrhosis and hepatocellular carcinoma is significantly elevated in these patients.
- Laboratory diagnosis of HDV is with enzyme immunoassay for HDV antibodies and HDV RNA by real-time polymerase chain reaction.

Therapy
- HDV is treated with pegylated interferon-α weekly for a minimum of 48 weeks.
- Newer therapies are currently under development.

Prevention
- Vaccination against hepatitis B (see Chapter 145) can be preventative because HDV needs HBV to replicate.

HEPATITIS DELTA VIRUS

Delta agent was identified by Mario Rizzetto in 1977 as a nuclear antigen distinct from hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg) in hepatocytes of some HBsAg carriers in Italy. It soon became clear that this passenger virus, termed *hepatitis D virus* (HDV), accompanied hepatitis B virus (HBV) infection and required the HBsAg for transmission. This unique HDV RNA genome is circular and resembles plant pathogens, including viroids and virusoids.[1] HDV is the only member of the genus *Deltavirus*.[2] In nature HDV is only found in patients who are also infected with HBV.

HDV is an enveloped virus of 36 nm that is distinct from 22-nm HBsAg or 42-nm HBV particles. Because HDV derives its viral envelope from HBV, the outer coat consists of the three forms (L, M, and S) of HBsAg and host lipids that surround the nucleocapsid, which includes the HDV genome in a complex with hepatitis delta antigen (HDAg). The genome is a small, single-stranded, circular RNA genome of approximately 1680 nucleotides, which has a high guanine-cytosine content, allowing it to fold into a rodlike structure.[3] Similar to other RNA viruses, there is no proofreading ability of the RNA polymerases, so the mutation rate is high, and the virus exists as a swarm of closely related quasispecies in an individual. The C-terminal region of the large-HDAg (L-HDAg) is the most variable.

Hepatitis Delta Antigen

The HDAg is the only known protein encoded by HDV and consists of two isoforms: small S-HDAg (24 kilodalton [kDa]) and large L-HDAg (27 kDa) (Fig. 146.1). Both are initiated from the same adenine-thymine-guanine (ATG) codon on the antigenomic RNA and are identical except that the L-HDAg has an additional 19 amino acids at the end of the C-terminus due to a unique RNA editing event during replication via the cellular enzyme adenosine deaminase acting on RNA 1 (ADAR1).[4–6] Each is translated from a distinct species of RNA. Both L- and S-HDAgs are phosphorylated and contain a nuclear localization signal that imports them into the nucleus.[7,8] L-HDAg acts as a dominant-negative inhibitor of replication and is necessary for binding HBsAg for virion assembly.[9] Binding of the L-HDAg to HBsAg is facilitated by prenylation of the C-terminal cysteine residue, which is a current target for new therapeutics (see "Treatment"). S-HDAg is involved in the initiation of RNA replication and is believed to have RNA chaperone activity.[10] Posttranslational modification of the S-HDAg affects the synthesis of genomic RNA and is a potential novel therapeutic target. Of note, L-HDAg and S-HDAg downregulate HBV replication through repression of two HBV enhancer regions and by activating the myxovirus resistance A (MxA) gene, which reduces export of HBV messenger (m)RNA from the nucleus.[11]

Hepatitis D Virus Life Cycle

Hepatocyte Entry

HDV infects only hepatocytes with no evidence of extrahepatic sites of its replication. The mechanism of HDV entry is similar to HBV because it requires L-HBsAg for entry through binding the sodium taurocholate cotransporting polypeptide receptor, followed by internalization through endocytosis (see "Attachment, Entry, and Hepatotropism" in Chapter 145). Once in the hepatocytes, the HDV nucleoprotein is released and translocated by HDAg to the nucleus via a nuclear localization signal.[3] The host range of HDV is limited to those species that can support the replication of hepadnaviruses and supply, in *trans*, the HBsAg envelope. For instance, HDV can infect woodchucks and can be packaged with HBsAg derived from woodchuck hepatitis virus. Chimpanzees are susceptible to HDV infection, and the infection is similar to that in humans.[12]

RNA Genome and Replication

The HDV RNA genome does not encode protein. Instead, the complementary antigenomic HDV RNA codes for HDAg and contains a sequence of approximately 85 nucleotides with an intrinsic ribozyme activity.[13,14] It is estimated that about 300,000 copies of genomic RNAs can be found in an infected hepatocyte during replication and 10-fold–less antigenomic RNA. S-HDAg is believed to be involved in RNA replication and may serve as an RNA chaperone.[15]

HDV replication involves the transcription of HDAg-encoding RNA from genomic RNA, the synthesis of antigenomic RNA from genomic RNA, and the synthesis of genomic RNA from antigenomic template via a double rolling circle mechanism (Fig. 146.2). Because HDV does

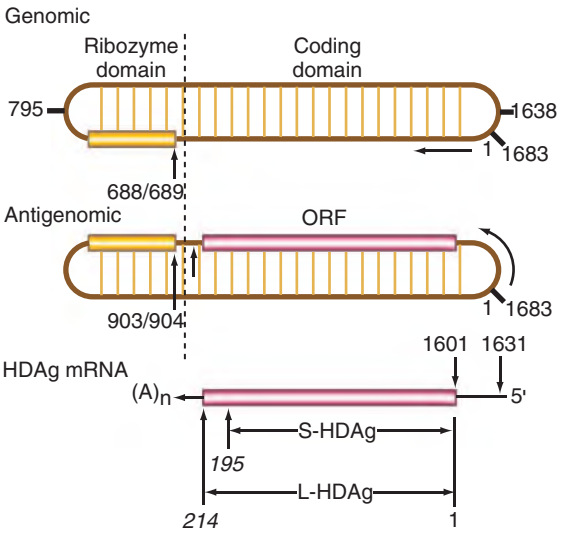

FIG. 146.1 Schematic structures of hepatitis D virus genomic and antigenomic RNA and hepatitis D antigen *(HDAg)*-encoding messenger RNA *(mRNA).* The minimum ribozymes are indicated by light boxes. The italicized numbers for HDAg are amino-acid residues. All other numbers are nucleotide positions on the genomic-sense RNA. The L-HDAg and S-HDAg are encoded by the antigenomic strand indicated by the open reading frame *(ORF). (Modified from MacNaughton TB, Lai MC. The molecular biology of hepatitis delta virus. In: Ou JE, ed.* Hepatitis Viruses. *Norwell, MA: Kluwer; 2002:109–128.)*

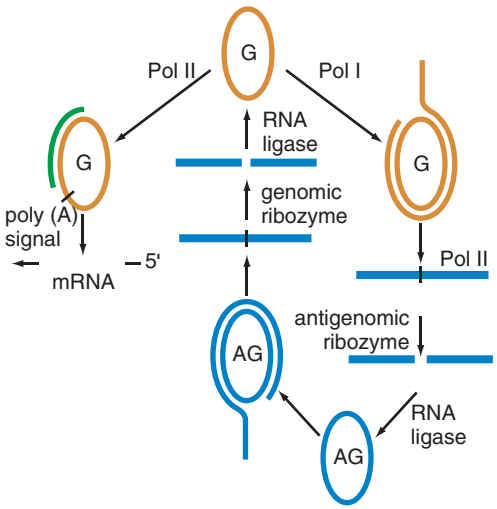

FIG. 146.2 Proposed model of hepatitis D virus RNA replication. The hepatitis D antigen-encoding messenger RNA *(mRNA)* is synthesized by polymerase II *(Pol II)* from the genomic RNA template independently of RNA replication. RNA Pol I synthesizes antigenomic RNA, and RNA Pol II synthesizes the genomic RNA. *AG,* Antigenomic strand; *G,* genomic strand. *(Modified from MacNaughton TB, Lai MC. The molecular biology of hepatitis delta virus. In: Ou JE, ed.* Hepatitis Viruses. *Norwell, MA: Kluwer; 2002:109–128.)*

not encode a polymerase, it recruits the host RNA polymerase II (Pol II) for transcription of HDV mRNA and for genomic RNA synthesis and likely uses host Pol II, and possibly also host Pol I, for antigenomic RNA synthesis.[10,16] Because of the rolling circle mechanism of RNA synthesis, multimeric linear transcripts are produced that are cleaved into monomers by the ribozyme activity. The linear product is ligated to form the antigenomic molecule, which then serves as a template for the synthesis of genomic RNAs through a rolling circle mechanism; HDV then interacts with the clamp of RNA Pol II, a cellular structure that holds RNA and DNA in place. HDAg loosens the clamp, which facilitates forward translocation of RNA Pol II while sacrificing fidelity.[17] HDV envelopment follows the scheme of virion assembly similar to HBV. Because HDV assembly can also occur in an L-HBsAg–independent fashion,[18] noninfectious HDV virions can be produced in abundance (≈90%). These particles are noninfectious because of the absence of pre-S1 domain, which is required for infectivity.

Pathogenesis

HDAg is the only viral protein known to be expressed during HDV infection. A high titer of immunoglobulin (Ig)M anti-HDV is strongly associated with elevated hepatitis D viremia and the severity of liver injury and alanine aminotransferase (ALT)/aspartate aminotransferase levels, whereas a more favorable course to HDV infection is found in individuals with IgG anti-HDV.[19] Although these antibody responses were present during acute and chronic infection, there was no association of IgM anti-HDV levels with HDV RNA, suggesting there is no protective role of anti-HDV antibodies. Woodchucks immunized with recombinant HDAg are only partially protected from subsequent challenge with HDV, which suggests that other mechanisms are responsible for immunity.[20]

The mechanisms of liver damage in HDV infection are unclear. There is conflicting evidence regarding whether HDV has a direct viral cytopathic effect that contributes to hepatocyte injury. In cell lines, expression of HDAg led to cytotoxic changes[21]; however, in transgenic mice, HDAg expression had no cytopathic effect.[22] HDV RNA levels have not been associated with severity of liver disease in 80 individuals with genotype 1 disease, supporting the concept that HDV does not directly lead to hepatocyte injury.[23] It is likely that the adaptive immune response plays a role in liver injury; however, the data are also conflicting. In one study HDV-specific T-cell responses correlated with lower ALT levels, suggesting that immune control of viral replication leads to lesser degrees of liver injury.[24] However, in another study histologic assessment demonstrated that the degree of cellular infiltration in the portal tracts and lobules correlates with the degree of staining for HDAg in the liver, suggesting that the immune response contributes to hepatocellular injury.[25] Thus the etiology of liver injury in HDV infection is unclear.

The adaptive immune response plays an important role in control of HDV infection, but the precise mechanisms are not understood. HDV-specific CD8 T-cell responses were detectable in those with previous infection but not in chronic, infection.[9,26] Another study demonstrated that chronic HDV was associated with a Th-2 response and secretion of interleukin-10. Taken together, these suggest that control of an HDV infection require Th1 CD4 and CD8 T-cell responses.

Epidemiology

An estimated 15 to 20 million people have serologic evidence of exposures to HDV, with an overall global prevalence of approximately 5% of HBV-infected persons.[27] Since the 1980s the prevalence of HDV has declined substantially as a result of better control of HBV due to more widespread vaccination practices and improved socioeconomic conditions. Infection with HDV has a worldwide distribution, although there are considerable geographic differences that do not entirely mirror the prevalence of HBV infection. Prevalence in the United States and Northern Europe are about 5% to 15% in HBsAg-positive carriers.[28] Immigrants from the Middle East, Africa, and Eastern Europe, and older patients from the Mediterranean Basin (most acquired parenterally), as well as young injection drug users account for the largest proportion of cases.[29–31] In the developing world, where HBV is highly endemic, such as Asia and sub-Saharan Africa, as many as two-thirds of the HBV-infected population is infected with HDV, although data in several countries are lacking. Some areas with the highest prevalence include parts of Central and West Africa (33% in Mauritania, 67% in Gabon), parts of the Amazon Basin (42%), the Middle East (33%–47%), India, Pakistan (24%–89%), and Northern Vietnam (15%–43%). In Mongolia greater than 60% to 88% of people with chronic hepatitis B have evidence of chronic HDV.[32] Higher HDV prevalence is also seen in patients with HIV-HBV coinfection compared with HBV monoinfection.[33] It should be noted that many of the estimated prevalence data are based on anti-HDV alone because commercial assays for HDV RNA are not readily available in many countries. In a recent study from Tanzania the prevalence of anti-HDV positivity was 5%; however, HDV RNA was not detectable in any of the anti–HDV-positive patients, and a

second serologic test (HDV-Ab kit; DiaPro, Milan, Italy) was negative in all the samples.[34]

There are eight HDV genotypes that have been identified by phylogenetic analyses. Divergence between genotypes ranges from 20% to 40%. Some of the genotypes are more aggressive than others.[35] HDV-1 strain is the most common worldwide, present in almost all continents. Four HDV-1 subgenotypes have been identified, and most have been associated with severe disease, including cirrhosis, fulminant hepatitis, and death. HDV-2 and HDV-4 strains are found in Asia and are associated with milder disease. HDV-3 is found in northern South America, especially the Amazon Basin, and is associated with the most severe form of HDV.[36,37] HDV-5 to HDV-8 are present only in Africa. HDV-5 is the next most common genotype after HDV-1 and originates from western and sub-Saharan Africa[35] (Fig. 146.3).

Diagnosis

HDV is diagnosed with detection of anti-HDV IgG, and because this remains positive with HDV clearance, chronic infection can be established using real-time polymerase chain reaction.[38] HDV IgM antibodies have a role if the HDV RNA is negative, and there is still a high suspicion for chronic HDV because viremia can fluctuate over time, and the variability of the HDV sequence can produce false-negative results.

Transmission and Clinical Manifestations

Similar to HBV, HDV is spread by blood, blood products, and bodily secretions and only requires a small inoculum to transmit.[9] Unlike HBV, perinatal transmission is uncommon. As a result of universal blood screening for HBsAg and widespread HBV vaccination, HDV has virtually disappeared in multiply transfused subjects and hemophiliacs in developed countries.[39] After exposure there is a short incubation period of 3 to 7 weeks. Because HDV requires the surface antigen from HBV, disease may occur as an acute coinfection with HBV or as a superinfection of a chronic HBV infection.

As with acute hepatitis B monoinfection, the clinical presentation and natural history of acute coinfection with HBV and HDV are highly variable. Acute coinfection usually occurs after an incubation period of 45 to 160 days. The incubation period during coinfection may display a biphasic pattern of ALT levels because the titers of the infecting inoculum for the two viruses may be different or because the two viruses are expressed sequentially.[12,40] In animal models the incubation period of each virus has been shown to be inversely proportional to the dose of the virus.[41] Usually the first episode is due to hepatitis B replication and immune response, followed by that of hepatitis D. Clinically, coinfection presents as an acute self-limited hepatitis with complete recovery with only 4% to 5% progressing to chronic HBV and HDV coinfection.[42–44] In rare instances a severe or fulminant hepatitis can occur.[45]

Superinfection of HDV in individuals with chronic hepatitis B generally results in acute severe hepatitis with a relatively short incubation period (2–8 weeks), followed by chronic hepatitis D in 90% of the cases. Of interest, when HDV clearance occurs after superinfection, there is increased HBsAg loss compared with HBV monoinfection.[46] Superinfection with HDV can be associated with fulminant hepatitis and chronic active hepatitis with cirrhosis. Fulminant hepatitis, a severe form of acute hepatitis, is 10 times more common in HBV-HDV coinfection than in chronic HBV infection alone. The prognosis with fulminant hepatitis is very poor. Massive liver necrosis can occur with mortality approaching 80% in the absence of liver transplantation. In patients with chronic hepatitis D, HDV is the dominant virus because the L-HDAg suppresses HBV replication (see "Hepatitis Delta Antigen"). Thus most HBV-HDV coinfected patients have low serum HBV DNA levels, and most are HBeAg negative.

Once chronic HDV infection is established, the clinical course of hepatitis is accelerated compared with HBV monoinfection. Cirrhosis occurs in 60% to 80% of chronic hepatitis D patients within 5 to 10 years, and the risk of hepatocellular carcinoma (HCC) is about threefold higher than in HBV monoinfection.[47–49] In one study where the HDV seroprevalence was 6%, the relative risk of cirrhosis and HCC was 2.58 and 2.87, respectively, in patients with both infections compared with those with HBV alone.[50] The high risk of HCC may reflect the higher rate of cirrhosis. Once cirrhosis is established, the risk for clinical decompensation is twofold higher than in HBV monoinfection.[47] Splenomegaly along with elevated aminotransferases and high levels of viremia are common in chronic hepatitis D.[47] However, unlike HBV, the level of HDV viremia does not seem to correlate with severity of disease.[48,51] The clinical course is affected by both the HDV genotype (see "Epidemiology") and the HBV genotype.[47,52] Liver disease is known to be accelerated in HIV-infected patients with triple coinfection (HIV/

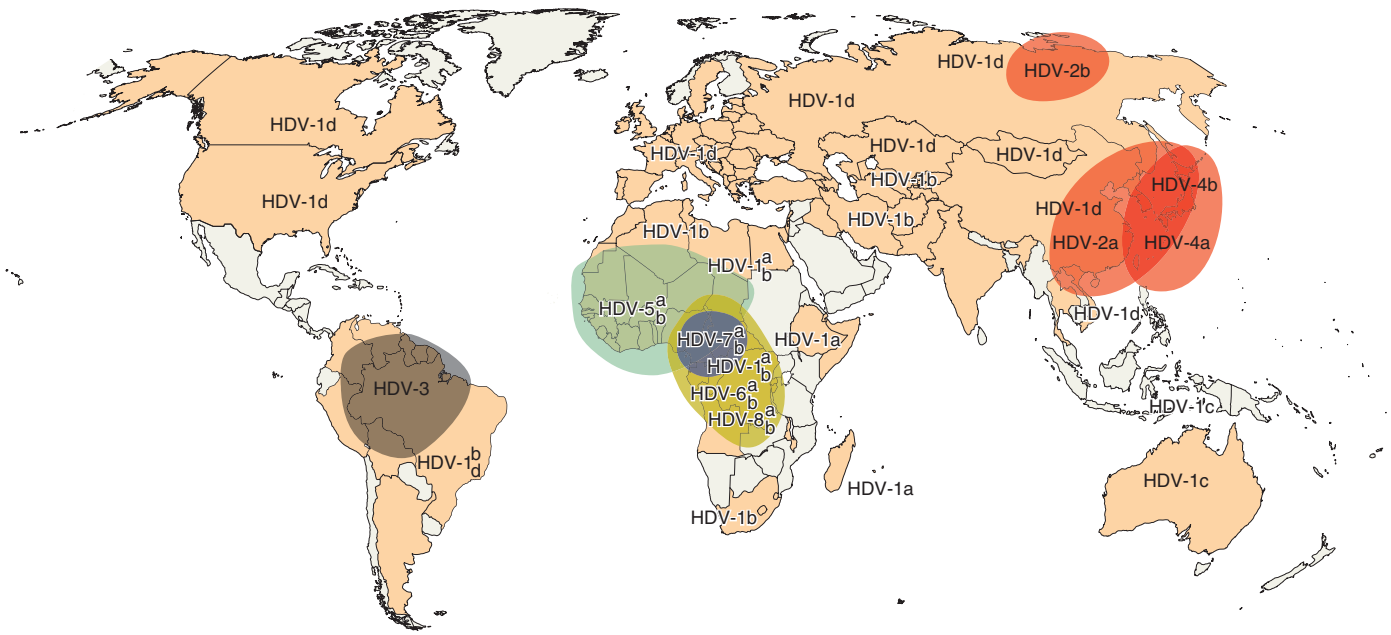

FIG. 146.3 Worldwide distribution of hepatitis D virus *(HDV)* genotypes and subgenotypes. *(From Le Gal F, Brichler S, Drugan T, et al. Genetic diversity and worldwide distribution of the* Deltavirus *genus: a study of 2,152 clinical strains.* Hepatology. *2017;66:1826–1841.)*

HBV/HDV), and the incidence of HCC and mortality is higher than in patients with HIV alone.[53,54]

Up to 30% of HBV-HDV coinfected individuals also have hepatitis C virus (HCV) antibodies; however, in one study 84% of them had undetectable HCV RNA, suggesting that HDV is the dominant virus in this triple infection.[55]

Treatment

Interferon-α (IFN-α) and pegylated (PEG) IFN-α have been shown to have antiviral activity against HDV. However, the efficacy of IFN-α in the treatment of HDV is limited unless high doses (9 million units three times a week) are used as in the reported studies. In one of the largest trials to date, treatment with IFN-α was only successful in achieving sustained HDV virologic response in 25% to 30% of individual patients.[56] With PEG IFN-α, sustained virologic response has been variable, occurring in 17% to 43% of patients in clinical trials. The current recommended treatment for chronic hepatitis D is weekly PEG IFN-α for a minimum of 48 weeks. The ultimate duration of therapy for HDV is still not known (also see Chapter 47), and late relapses have been reported.[57] In the long term, biochemical and histologic improvements with IFN-α can occur, even among those who relapse. In a previous study, improvement in liver histology was maintained 10 years post-treatment among the patients who received high-dose IFN-α.[58] Nucleoside reverse-transcriptase inhibitors with activity against HBV, including lamivudine and entecavir, have been shown to be ineffective at controlling HDV replication, which is not surprising because HDV does not have its own polymerase.[59] HDV RNA declines, and virologic suppression have been reported in HIV/HBV/HDV coinfected patients receiving long-term tenofovir DF, correlating in one study with declines in HBsAg and reduction in fibrosis.[60] This decline may have been related to the longer duration of therapy that led to decrease in HBsAg.

Newer therapies are under development and fall into three categories: entry inhibitors, farnesyltransferase inhibitors, and nucleic acid polymers. Blocking HDV entry with Myrcludex B ± PEG IFN-α has shown promise in phase II clinical trials.[61] Farnesyltransferase inhibitors (lonafarnib) prevent virion assembly, and in early phase II clinical trials, a reduction in HDV RNA was observed over 28 days.[62] Longer studies up to 24 weeks with and without ritonavir as a boosting agent and/or PEG IFN-α have led to HDV RNA suppression at the end of treatment but not necessarily off treatment. Gastrointestinal toxicity is a dose-limiting side effect of lonafarnib. The mechanism of action of nucleic acid polymers is believed to be decreasing viral secretion from hepatocytes. One example is intravenous REP 2139 (Replicor; Montréal, Quebec, Canada), which was given in combination with PEG IFN-α to 12 HBV-HDV coinfected patients in a phase II clinical trial.[63] Patients received 30 weeks of REP 2139 and 48 weeks of PEG IFN-α with a 15-week overlap of the two drugs. One year after the end of treatment, 7 were HDV RNA negative and 8 were HBV DNA negative. Four individuals had a serious adverse event, which was attributed to the PEG IFN-α, but only 1 discontinued therapy. Other treatments under investigation include PEG IFN-γ, which is being tested at two doses for 48 weeks in 33 individuals. Interim 24-week results from 10 of the 33 participants showed that five had a ≥2-log decline in HDV RNA, and 4 became HDV RNA negative.[64] Further studies with these and other agents in development are needed to determine if they are superior to PEG IFN-α.

Transplantation

Liver transplantation is reserved for patients with end-stage liver disease from HBV-HDV coinfection. Reinfection of the graft is prevented with administration of hepatitis B immunoglobulin because prevention of HBV reinfection stops propagation of HDV. There is no evidence for latency of HDV outside the liver. The 5-year survival of liver transplantation for HDV is >80%.

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