

Reference 3

Including  
Expanded Liver  
Transplant  
Section

# SCHIFF'S DISEASES OF THE LIVER

TENTH EDITION

VOLUME ONE

EUGENE R. SCHIFF  
MICHAEL F. SORRELL  
WILLIS C. MADDREY

 Lippincott Williams & Wilkins  
a Wolters Kluwer business

*Executive Editor:* Charles W. Mitchell  
*Senior Managing Editor:* Lisa Kairis  
*Marketing Manager:* Angela Panetta  
*Project Manager:* Bridgett Dougherty  
*Senior Manufacturing Manager:* Benjamin Rivera  
*Creative Director:* Doug Smock  
*Compositor:* Laserwords Private Limited, Chennai, India  
*Printer:* RR Donnelley

© 2007 by LIPPINCOTT WILLIAMS & WILKINS, a Wolters Kluwer business  
530 Walnut Street  
Philadelphia, PA 19106 USA  
LWW.com

9th edition © 2003 Lippincott Williams & Wilkins, 8th edition © 1999 Lippincott Williams & Wilkins, 7th edition © 1993 JB Lippincott Co, 6th edition © 1987 JB Lippincott Co, 5th edition © 1982 JB Lippincott Co, 4th edition © 1975 JB Lippincott Co, 3rd edition © 1969 JB Lippincott Co, 2nd edition © 1963 JB Lippincott Co, 1st edition © 1956 JB Lippincott Co

All rights reserved. This book is protected by copyright. No part of this book may be reproduced in any form or by any means, including photocopying, or utilized by any information storage and retrieval system without written permission from the copyright owner, except for brief quotations embodied in critical articles and reviews. Materials appearing in this book prepared by individuals as part of their official duties as U.S. government employees are not covered by the above-mentioned copyright.

Printed in the USA

---

#### Library of Congress Cataloging-in-Publication Data

Schiff's diseases of the liver. — 10th ed. / edited by Eugene R. Schiff, Michael F. Sorrell, Willis C. Maddrey.

p. ; cm.

Includes bibliographical references and index.

ISBN 13: 978-0-7817-6040-9

ISBN 10-7817-6040-2

1. Liver—Diseases. I. Schiff, Eugene R. II. Sorrell, Michael F. III. Maddrey, Willis C. IV. Title: Diseases of the liver.

[DNLM: 1. Liver Diseases. WI 700 S333 2007]

RC845.D53 2007

616.3'62—dc22

2006023630

---

Care has been taken to confirm the accuracy of the information presented and to describe generally accepted practices. However, the authors, editors, and publisher are not responsible for errors or omissions or for any consequences from application of the information in this book and make no warranty, expressed or implied, with respect to the currency, completeness, or accuracy of the contents of the publication. Application of this information in a particular situation remains the professional responsibility of the practitioner.

The authors, editors, and publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accordance with current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new or infrequently employed drug.

Some drugs and medical devices presented in this publication have Food and Drug Administration (FDA) clearance for limited use in restricted research settings. It is the responsibility of the health care provider to ascertain the FDA status of each drug or device planned for use in their clinical practice.

To purchase additional copies of this book, call our customer service department at (800) 638-3030 or fax orders to (301) 223-2320. International customers should call (301) 223-2300.

Visit Lippincott Williams & Wilkins on the Internet: at LWW.com. Lippincott Williams & Wilkins customer service representatives are available from 8:30 am to 6 pm, EST.

10 9 8 7 6 5 4 3 2 1

# Hepatitis B and D

ANNA S.F. LOK, HARI S. CONJEEVARAM, FRANCESCO NEGRO

## KEY CONCEPTS

- a • There are approximately 400 million hepatitis B carriers worldwide and 1.25 million carriers in the United States. The prevalence of hepatitis B virus (HBV) infection is related to the predominant mode of transmission and the age at infection.
- b • Acute HBV infection may manifest as subclinical hepatitis, icteric hepatitis, or fulminant hepatitis. Chronic HBV infection may manifest as inactive carrier state, chronic hepatitis, cirrhosis, or hepatocellular carcinoma (HCC).
- Many lines of evidence support an etiologic association between chronic HBV infection and HCC.
- c • The aims of antiviral treatment of chronic hepatitis B are to suppress HBV replication, induce remission in liver disease, and prevent the development of cirrhosis and (HCC).
- Approved treatments of chronic hepatitis B include interferon- $\alpha$  (standard and pegylated), lamivudine, adefovir, and entecavir.
- Hepatitis D virus is dependent on HBV. Hepatitis D occurs as coinfection with HBV or superinfection in persons with chronic HBV infection.

## Epidemiology

Hepatitis B infection is a global public health problem. It is estimated there are approximately 400 million hepatitis B virus (HBV) carriers in the world, of whom over 500,000 die annually from hepatitis B–associated liver disease (1). In the United States, an estimated 1.25 million individuals are chronically infected with HBV (2,3). Hepatitis B carrier rate varies from 0.1% to 20% in different areas of the world (Table 29.1). The wide range in carrier rate is related to differences in the predominant mode of transmission and the age at infection. Understanding the epidemiology of hepatitis B is important in the implementation of vaccination programs for the prevention of HBV infection.

### PREVALENCE

The prevalence of HBV infection varies in different geographical areas (Table 29.1) (4). In low prevalence

areas such as the United States, western Europe, Australia and New Zealand, the hepatitis B surface antigen (HBsAg) carrier rate is approximately 0.1% to 2%. In intermediate prevalence areas like the Mediterranean countries, Japan, India and Singapore, the carrier rate is approximately 3% to 5%. In high prevalence areas such as Southeast Asia and sub-Saharan Africa, the carrier rate is 10% to 20%. The prevalence of current and past HBV infection is estimated to be 5% in the United States and close to 100% among adults in some parts of Southeast Asia and Africa. In general, there is an increasing prevalence of HBV infection with age. Within the United States, the prevalence of HBV infection is higher among African Americans, Hispanics and Asians than in the white population (3). Several communities have been reported to have higher carrier rates than their neighboring regions, namely, Alaskan Eskimos, Asian-Pacific Islanders and Australian Aborigines.

In most high prevalence areas such as Hong Kong and China, perinatal transmission is the major mode

**TABLE 29.1. PATTERNS OF HEPATITIS B VIRUS INFECTION**

Prevalence	High	Intermediate	Low
Carrier rate	8%–20%	3%–7%	0.1%–2%
Geographical distribution	Southeast Asia China Pacific Islands Sub-Saharan Africa Alaska (Eskimos)	Mediterranean basin Eastern Europe Central Asia Japan Latin and South America Middle East Early childhood	United States and Canada Western Europe Australia New Zealand
Predominant age at infection	Perinatal and early childhood		Adult
Predominant mode of transmission	Maternal–infant Percutaneous	Percutaneous Sexual	Sexual Percutaneous

of spread accounting for 40% to 50% of chronic HBV infection (5,6). However, horizontal spread during the first 2 years of life is the major mode of transmission in other endemic areas including Africa and the Middle East (7,8). The exact reason for the preponderance of perinatal transmission among Orientals is not clear but is at least in part related to the high prevalence of hepatitis B e antigen (HBeAg) among Asian carriers of reproductive age—40% to 50% versus 10% to 20% among African carriers of the same age-group (6,9). In intermediate prevalence areas, transmission occurs among all age-groups but early childhood infection accounts for most cases of chronic infection. In low prevalence areas, most infections are acquired in early adult life through unprotected sexual intercourse or intravenous drug abuse. The age at infection has a significant impact on the clinical outcome because chronic infection occurs in approximately 90% of infants infected at birth, in 25% to 50% of children infected between the age of 1 and 5 years, and in less than 5% of those infected during adult life (5,10–12).

## MODE OF TRANSMISSION

### Transfusion

In the 1960s, the risk of hepatitis B infection from transfusions of commercial blood was as high as 50% and HBsAg was detected in up to 60% of patients with post-transfusion hepatitis. The exclusion of paid donors and the application of hepatitis B serologic screening in the 1970s dramatically reduced the incidence of post-transfusion HBV infection (13). Currently, approximately 80 cases of transfusion-associated HBV infection are reported in the United States each year (14). In the United States, both HBsAg and hepatitis B core antibody (anti-HBc) are used for blood donor screening. Anti-HBc was initially used as a surrogate marker for non-A non-B hepatitis virus. Anti-HBc has been retained after the implementation of hepatitis C testing to detect donors who are in the window phase during recovery from acute hepatitis B or who

have low level chronic HBV infection. The practical value of anti-HBc screening is not clear because of the possibility of false-positive test result, the low incidence of transfusion-associated HBV infection with HBsAg screening only in low prevalence areas, and the need to exclude as many as 22% of the donor population in high prevalence areas (15–18). Currently, the risk of transfusion related hepatitis B from blood donors who test negative for HBsAg and anti-HBc is estimated to be 1 in 63,000 (range 1:30,000 to 1:250,000 episodes per unit transfused) (19).

Recently there have been debates on serology versus nucleic acid testing (NAT) of blood donors. A recent review concluded that NAT for HBV will probably detect only a few more donor units that may be associated with risk of transmitting HBV infection compared to serologic screening for HBsAg and anti-HBc (20). With the current estimated 13 million donations per year in the United States and 1.8 transfused components per donation, introduction of NAT would be expected to prevent 30 to 35 HBV-containing transfusions per year. Because of the low rates of viral persistence and clinical disease following HBV transmission in the setting of seronegative blood transfusion, the clinical impact and cost-effectiveness of NAT is expected to be low. NAT of whole blood was estimated to avert 9 to 37 HBV infections at an additional cost of US \$39 to 130 million per year (21).

### Percutaneous transmission

Percutaneous inoculation of blood or body fluid plays a major role in the transmission of hepatitis B infection. Needle sharing by intravenous drug users is an important route of transmission of hepatitis B. Reuse of contaminated needles for tattoos, acupuncture and ear piercing also provide opportunities for percutaneous transmission.

### Sexual transmission

In the United States and many developed countries, sexual transmission is the most important mode of spread

of HBV. The Centers for Disease Control and Prevention reported that sexual transmission accounts for almost 50% of acute HBV infection among individuals in whom data on risk factors were available (22). A high prevalence of chronic HBV infection has been reported in men who have sex with men as well as in heterosexuals with multiple sex partners. The annual incidence of new HBV infections among homosexual men decreased significantly in the 1980s as a result of education on safe sex practice to prevent human immunodeficiency virus (HIV) infection (23). However, recent reports in the United States suggest that both heterosexual transmission and transmission among homosexual men are on the rise (22). The risk of sexual transmission of HBV infection is proportional to the number of lifetime sex partners, low education level, paid sex, and history of sexually transmitted diseases.

### Perinatal transmission

The rate of neonatal HBV infection from an infected mother is less than 10% in Western countries. Nonetheless, an estimated 20,000 infants are born to HBsAg carrier women in the United States annually (24). In areas with high endemicity such as China, perinatal infection is the most common mode of transmission. The risk of maternal–infant transmission is related to the HBV replicative status of the mother. The risk is 85% to 90% for infants born to HBeAg-positive mothers and 30% for infants born to HBeAg-negative mothers (25). More recent studies demonstrated that maternal serum HBV DNA levels correlate better with the risk of transmission (26). Maternal–infant transmission takes place at the time of delivery by maternal–fetal transfusion or exposure to maternal blood during passage through the birth canal and postnatally through intimate mother–baby contact. Intrauterine transmission is uncommon as HBsAg is detected in infants much later. In addition, passive–active immunization at birth has been demonstrated to have an efficacy rate of more than 90% in the prevention of HBV infection (27). Cesarean section has not been shown to eliminate the risk of perinatally acquired HBV infection (28) and should not be routinely recommended for carrier mothers. Although HBsAg can be detected in breast milk, there is no evidence that HBV infection can be transmitted by breast-feeding (29); infants born to carrier mothers may be breast-fed if they have been vaccinated. The risk of transmission during amniocentesis is also low (30). Universal vaccination of all newborns and additional administration of hepatitis B immune globulin (HBIG) to those who are born to carrier mothers were initiated in many Southeast Asian countries in the 1980s. These programs have led to significant reduction in HBsAg carrier rate as well as decrease in the incidence of hepatocellular carcinoma (HCC) among children (31).

### Health care environment

HBV is the most commonly transmitted blood-borne virus in the health care setting (32). Transmission generally occurs from patient to patient or from patient to health care personnel via contaminated instruments or accidental needle stick injury. The risk of acquiring HBV infection after needle stick injury is related to the HBeAg status of the source patient. There have been several outbreaks of hepatitis B infection in the health care environment. One report involved transmission from a patient with diabetes to another through the contaminated platform of a spring-loaded lancet device for finger sticks (33). Outbreaks of HBV infection were also reported in several hemodialysis units as a result of failure to identify and isolate patients who were infected and to vaccinate those who were susceptible (34). Transmission of HBV infection from health care workers to patients is rare. One outbreak was traced to a cardiothoracic surgeon despite no identified flaws in precautions on infection control during operations (35). Transmission was thought to be related to tears in the gloves and minor cuts on the surgeon's fingers during prolonged suturing. Nosocomial transmission can be prevented by screening of blood and blood products, use of disposable needles and equipment, proper sterilization of surgical instruments, enforcement of infection control measures, and vaccination of health care workers.

In many developed countries, guidelines have been established to define the parameters within which health care workers with hepatitis B can operate. In the United States, health care workers who are HBeAg positive are restricted from performing invasive procedures (36,37). The Centers for Disease Control and Prevention recommends that health care workers with HBV infection should not perform exposure prone procedures unless they have sought counsel from an expert review panel and have been advised on the circumstances under which they may perform such procedures. The difference in the scope of permissible work between HBeAg-positive and HBeAg-negative carriers is related to the traditional concept that HBeAg is a reliable marker of infectivity. However, a recent report found that transmission of HBV infection occurred from four HBeAg-negative surgeons. These surgeons had detectable HBV DNA in serum and were infected with precore stop codon variants (38). This and other similar incidents have led to the proposal that serum HBV DNA levels be used to categorize the infectivity of health care workers but it is also known that serum HBV DNA levels can fluctuate and may be intermittently undetectable in patients with chronic HBV infection (37,39,40). As vertical transmission is rarely documented with maternal HBV DNA levels below  $10^7$  copies/mL, it is thought that transmission of HBV

via needle-stick injury is also unlikely to occur at HBV DNA levels below  $10^7$  copies/mL (39). It has been proposed that health care workers with higher HBV DNA levels receive antiviral therapy to enable them to return to work without risking nosocomial infection.

### Hemodialysis patients

Patients with renal failure on hemodialysis may be infected through blood transfusions, contamination of dialysis machines or equipment, as well as interpersonal horizontal transmission in the dialysis units. Improved infection control and the availability of vaccines have reduced the incidence of HBV infection among hemodialysis patients from 3% in 1980 to 0.1% in 1993 in the United States and has remained stable in the past decade (34,41). However, dialysis patients have impaired antibody response to vaccines. Therefore, vigilance is still needed to prevent outbreaks.

In a recent survey of all US chronic hemodialysis centers (41) the percent of patients vaccinated against HBV infection increased from 47% to 56% and the percent of staff vaccinated increased from 87% to 90% between 1997 and 2002. Although the overall incidence of HBV infection did not correlate with the infection control practices, it was noted that the incidence of HBV infection in 2002 was higher among patients in centers where injectable medications were prepared on a medication cart compared to a dedicated medication room.

A possible contributing factor for continued transmission of HBV infection in adult hemodialysis units appears to be the presence of occult HBV infection (serum HBsAg negative but HBV DNA positive). In a recent study of 241 adult hemodialysis patients in a North American urban center (42), only two patients (0.8%) were HBsAg-positive but nine (4%) HBsAg-negative patients were HBV DNA positive.

### Transplantation

Currently, organ donors are routinely screened for HBsAg. Transmission of HBV infection has been reported after transplantation of extrahepatic organs such as kidneys from HBsAg-positive donors. This may be related to residual blood in the vascular pedicles due to inadequate flushing or the presence of infectious virions in the kidneys. Transmission of HBV infection has also been reported after transplantation of avascular tissues such as cornea (43).

The role of anti-HBc testing in organ donor screening is uncertain because of the possibility of false-positive results, the potential loss of up to 5% of donors even in low endemic areas (44), and the uncertainty about the infectivity of organs from donors who have isolated anti-HBc (45). The incidence of HBV infection from donors with isolated anti-HBc is very low (0% to

2%) in heart and kidney recipients but varies from 0% to 78% in liver recipients (44,46–48). A recent study found that the estimated probability of undetected hepatitis B viremia is higher among tissue donors compared to first-time blood donors and the addition of NAT to the screening of tissue donors is expected to reduce the risk of HBV infection (49).

### Others

In endemic areas, horizontal transmission among children may result from close bodily contact leading to transfer of virus across minor skin breaks and mucous membranes. Blood-feeding insects like mosquitoes have been demonstrated to serve as vectors for HBV transmission in animal models but firm evidence for this mode of transmission in humans is lacking. Various body secretions have been reported to test positive for HBsAg but only semen and saliva have been consistently shown to harbor infectious virions (50,51). Although HBV DNA has been detected in the saliva of some hepatitis B carriers, there is no convincing evidence that hepatitis B can be transmitted orally (52,53). As HBV survives for a long time outside the human body, transmission via contaminated environmental surfaces and daily articles such as toothbrushes, razors, eating utensils or even toys may also be possible.

### HIGH-RISK GROUPS

Health care workers have a higher hepatitis B carrier rate than the general population. The prevalence is particularly high among surgeons, pathologists, and physicians working in hemodialysis and oncology units. Apparent skin breaks, minor cuts and accidental needle stick injuries serve as portals of entry. Other health care workers having increased risk of HBV infection include dentists and laboratory personnel who have contact with serum. Institutionalized mentally handicapped persons as well as their attendants and family members also have a high rate of hepatitis B infection. Despite screening of blood products, patients requiring frequent transfusion of blood or blood products—those with thalassemia and hemophilia—have an increased risk of contracting hepatitis B infection. Other high-risk groups include intravenous drug users particularly those who share syringes, men who have sex with men and promiscuous heterosexuals, immigrants from HBV endemic countries, and spouses, sexual partners, and household members of HBV carriers.

### CHANGING EPIDEMIOLOGY

The worldwide incidence of HBV infection is decreasing (1,22). Mass vaccination for newborns and catch-up vaccination for children and adolescents play a major role in reducing HBV infection among infants and

j

k

children. Increased public awareness of hepatitis, educational campaigns to prevent HIV infection leading to modification of high risk sexual behavior, and reduction of syringe sharing among intravenous drug users have contributed to the decrease in HBV infection among adults.

In the United States, the incidence of acute hepatitis B has significantly declined over the past decade. According to the Centers for Disease Control and Prevention, the incidence of acute hepatitis B during the years 1990 to 2002 has declined from 8.5 per 100,000 population to 2.8 per 100,000 population (22); the most significant decline was seen among ages 0 to 19 years (rate of 3.0 to 0.3). The decline was more marked in women compared to men. However, incidence has remained the same if not increased among certain adult groups: Those with multiple sexual partners, men who have sex with men, and injection drug users. Sexual transmission among susceptible individuals remains a significant risk factor for hepatitis B transmission in the United States. This is in part related to lack of resources and infrastructure for vaccination of adults as well as missed opportunities. In a recent study of 833 men who have sex with men, aged 15 to 29 years, 44% were susceptible to HBV infection; most of these men were found to be either unaware of protective vaccines, had never been offered vaccination, or perceived themselves at low risk (54).

Another important aspect of HBV epidemiology is that in many developed countries, immigrants from countries that are endemic for HBV infection now constitute an increasing proportion of those with chronic HBV infection (55). In addition, some studies also showed that these immigrants have a higher incidence of acute HBV infection (56). These and other studies (57) suggest that screening and immunization of susceptible adults along with immunization of children (especially if they were born in countries where universal vaccination is not in place) whose parents immigrated from HBV endemic countries may be of great importance in controlling HBV infection in developed countries.

## Vaccination

### INDICATIONS

Vaccination against hepatitis B remains the mainstay of prevention. Universal vaccination of all newborn or at least newborn of all HBV-infected mothers is currently practiced in most countries throughout the world. The World Health Organization (WHO) has recommended that combination of hepatitis B and childhood vaccines be used where possible, to reduce the logistic costs of vaccine delivery especially in areas where it is most needed. However, due to decreased immunogenic potential of other vaccines especially during the

**TABLE 29.2. INDICATIONS FOR HEPATITIS B VACCINE**

1. All newborns<sup>a</sup>
2. All children and adolescents not vaccinated at birth
3. High-risk adults:
  - a. Health care workers
  - b. Men who have sex with men
  - c. Persons with multiple sexual partners
  - d. Injection drug users
  - e. Patients on hemodialysis
  - f. Institutionalized patients
  - g. Health care workers and public safety workers
  - h. Spouse, sexual partners and household members of HBV carriers

<sup>a</sup>For infants born to carrier mothers, hepatitis B immune globulin (HBIG) is also administered at birth.

initial 6 weeks after birth, it is currently recommended that only monovalent vaccines should be administered to the newborn. In some developed countries, where universal vaccination of all newborn is not in place, vaccination of adolescents to prevent sexual transmission is implemented. Vaccination of adults is recommended for high risk groups including health care workers, men who have sex with men, persons with multiple sex partners, injection drug users, sex partners, and household members of HBV carriers, public safety workers, institutionalized patients, and patients on chronic hemodialysis (Table 29.2) (58).

### ADMINISTRATION SCHEDULE

There are two types of hepatitis B vaccines, plasma-derived and recombinant, the latter is currently used in most countries. Recombinant HBV vaccines consist of HBV small S protein (HBsAg) produced by yeast or mammalian cells. Hepatitis B vaccine is usually administered intramuscularly in three doses at 0, 1 and 6 months, the dose being 10 to 20 µg in adults and 5 to 10 µg in children (Table 29.3). For adults, the injections are given in the deltoid muscle, whereas in newborns and young children the recommended site is the anterolateral thigh. In patients with hemophilia,

**TABLE 29.3. HEPATITIS B VACCINES AND DOSAGE RECOMMENDATIONS**

Vaccine brand	Age-group (y)	Dose (µg)	Volume (mL)	Number of doses
Engerix-B	0–19	10	0.5	3
	≥20	20	1.0	3
Recombivax HB	0–19	5	0.5	3
	≥20	10	1.0	3
(Optional two doses)	11–15	10	1.0	2

For hemodialysis patients, recommended dose is 40 µg with each dose (Engerix-B 40 µg/2.0 mL and Recombivax HB dialysis formulation 40 µg/1.0 mL).

it is recommended to administer the vaccine subcutaneously.

For infants born to HBsAg-negative mothers and unvaccinated children/adolescents up to 19 years of age, 3 doses (0, 1 and 6 months) of vaccine at half strength should be administered. For adults 20 years and older, the same regimen is implemented using full dose (10 µg of Recombivax HB and 20 µg of Engerix-B). An alternative two-dose schedule had been approved for adolescents.

For newborns of HBsAg carrier mothers, HBIG 0.5 mL and the first dose of vaccine should be administered at birth, using different sites. Combination of HBIG and hepatitis B vaccine has been shown to be 95% efficacious in preventing perinatal transmission of HBV infection (27,59,60).

For patients on hemodialysis or immunocompromised patients, higher doses of vaccine are needed: 40 µg of Recombivax HB or Engerix-B. Anti-HBs titer should be monitored annually, and booster doses administered when hepatitis B surface antibody (anti-HBs) titer falls below 10 IU/L.

Follow-up testing for protective antibodies is recommended for individuals who continue to be at risk including infants born to HBsAg-positive mothers, health care workers, hemodialysis patients, and sexual partners of HBsAg carriers (58).

Some vaccines have also incorporated pre-S1 (large S) and/or pre-S2 (middle S) proteins to increase the immunogenicity but these vaccines are not available in most countries.

## EFFICACY

A protective response defined as an anti-HBs titer more than 10 IU/L is achieved in approximately 95% of vaccine recipients. Several studies have shown that vaccination is effective in inducing protective immunity and in preventing HBV infection even in men who have sex with men, (61–63) and newborns of carrier mothers (27,59,64). In countries where the prevalence of HBeAg among carrier mothers is low, it has been shown that the vaccine alone has similar efficacy in preventing HBV infection as a combination of vaccine and HBIG in preventing perinatal infection (64). Although this approach can be cost saving, it may not be adequate in countries where the prevalence of HBeAg among carrier mothers is high or in countries where a high percent of HBeAg-negative mothers have high serum HBV DNA levels.

## FACTORS ASSOCIATED WITH NONRESPONSE AND MANAGEMENT OF VACCINE NONRESPONDERS

Approximately 2.5% to 10% of vaccine recipients fail to respond with adequate anti-HBs titers after one

course of HBV vaccine. The reasons for nonresponse are several and include older age, obesity, chronic medical illnesses such as renal failure, diabetes, cirrhosis, immunosuppression such as patients with HIV infection or organ transplantation, and technical problems such as intragluteal injection and inadvertent freezing of the vaccines.

Nonresponse to HBV vaccine has been reported to be associated with impaired lymphocyte activation as well as genetic factors including certain human leukocyte antigen (HLA) class II genes such as HLA-DRB1\*0301 and cytokine gene polymorphisms (65–67).

For individuals who failed to respond after a full course of vaccination the recommendation is to repeat another course of vaccine. If a person still remains a nonresponder, further vaccination is usually not effective but most of these individuals can mount an adequate immune response upon infection because exposure to HBV stimulates both T and B cell responses to HBsAg as well as hepatitis B core antigen (HBcAg). Nonresponders to two courses of vaccine should be tested for HBsAg as some may be undiagnosed carriers.

## DURABILITY OF VACCINE RESPONSE AND NEED FOR BOOSTERS

Several studies showed that 30% to 66% of individuals had protective levels of anti-HBs ( $\geq 10$  mIU/mL) for even 15 years or more after receiving plasma derived HBV vaccines and 90% had anamnestic response after booster vaccination (68–71). Breakthrough infections appear to occur mostly among those who did not have an initial response to vaccination (70). Two recent studies found that persistence of anti-HBs response up to 18 years after administering plasma-derived and recombinant vaccines was comparable (68,72).

Although anti-HBs titers decline with time, the incidence of HBV infection among individuals who were vaccinated at birth is low and there is no consensus on the need for booster vaccination. The European Consensus Group on hepatitis B Immunity in 2000 recommended that booster doses be considered in those who are immunocompromised or at a high risk of exposure (73). A recent report of the Steering Committee for the prevention and control of infectious diseases in Asia (74) recommended booster vaccination approximately 10 to 15 years after primary vaccination especially among children vaccinated as infants; when monitoring of antibody levels is not feasible; in all immunocompromised patients with anti-HBs levels below 10 mIU/L; and for health care workers in endemic countries. By contrast, a Viral Hepatitis Prevention Board that convened in 2004 concluded that existing data do not support the need for booster doses in universal HBV immunization programs, but the risk of



infection through sexual or occupational exposure later in life on those vaccinated as neonates is unknown (75).

## IMPACT OF HEPATITIS B VIRUS VACCINATION

HBV vaccination has been shown to reduce the incidence of acute HBV infection and HCC, and the prevalence of chronic HBV infection (31,57,76,77). HBV vaccine is the first vaccine that has been shown to prevent cancer (HCC) in humans. After the implementation of a nationwide vaccination program for newborn and children in 1984, the carrier rate among children decreased from 10% in 1984 to less than 1% in 1999 (31) while the incidence of HCC declined from 0.79 cases per 100,000 between the years 1981 and 1986 to 0.36 cases between the years 1990 and 1994 (77). In the United States, universal vaccination of all newborns was implemented in 1991 and it was expanded to include vaccination of all adolescents aged 11 to 12 years in 1995, and children aged less than 18 years, who had not been vaccinated previously, in 1999. This has resulted in a significant 89% reduction of acute hepatitis B in children and adolescents during 1990 to 2002 (22).

## SAFETY OF HEPATITIS B VACCINATION

The safety of hepatitis B vaccination has been well established. The most common adverse reaction is soreness over the injection site. Other adverse reactions include low-grade fever, malaise, headache, arthralgia and myalgia. Hepatitis B vaccines have no teratogenic effects and can be administered during pregnancy (78,79).

There has been concern about the possibility of hepatitis B vaccine leading to the development of demyelinating central nervous system diseases including multiple sclerosis (80,81) and also Guillain-Barre syndrome (82). It has been speculated that these "adverse reactions" could be related to "molecular mimicry." However, many studies have failed to show a statistically significant temporal or causal association between HBV vaccine and these neurologic or immunologic conditions (83–87). Because of concerns about mercury exposures, current preparations of HBV vaccines do not contain thimerosal as a preservative.

Based upon current evidence and the proven benefit of hepatitis B vaccine, the WHO has recommended that all countries continue their hepatitis B vaccine programs (88).

## SPECIAL SETTINGS

### Isolated antihepatitis B core individuals

The presence of an isolated anti-HBc does not always denote prior exposure to HBV infection. HBV vaccination has been recommended to differentiate those

who had prior exposure from those with false-positive anti-HBc test results (15). With improved specificity of current anti-HBc assays, most individuals with isolated anti-HBc have genuinely positive test results and do not need to be vaccinated but there is no harm if vaccine is administered.

### Patients on chronic hemodialysis

Response to HBV vaccine is impaired in patients with renal failure. A recent report from the Cochrane group found that there was no difference in response between plasma derived and recombinant HBV vaccines (89). Response to HBV vaccine was similar in hemodialysis versus peritoneal dialysis patients (90).

### Patients with chronic liver disease

Hepatitis B vaccination along with vaccination against hepatitis A is currently recommended for all patients with underlying chronic liver disease. Acute hepatitis B superimposed on chronic hepatitis C has been reported to be associated with increased risk of liver failure (91). Immune response to HBV vaccines among patients with chronic liver disease varies from 70% to 90% (91). In general, response rates are similar to healthy subjects with no liver disease except in patients with cirrhosis but response rates are substantially lower (<50%) in patients with decompensated cirrhosis awaiting liver transplantation (92,93). Therefore, it is recommended that HBV vaccination should be administered early, before a patient develops cirrhosis.

### Patients with human immunodeficiency virus infection

Several reports have suggested that patients with HIV infection have a blunted response to HBV vaccine compared to HIV-negative individuals. A recent large randomized, double-blind study of two doses of recombinant HBV vaccine (standard dose of 20  $\mu$ g and double dose of 40  $\mu$ g) (94) showed that a response was seen in 34% and 47% in the standard and double dose groups, respectively. Response rates were higher in those with high CD4 count and low HIV RNA level.

### Novel methods of vaccine administration

In an effort to reduce the number of injections and increase compliance, several combination vaccines have been developed. They include combinations of hepatitis A and B vaccine for adults and children, and a hexavalent combined vaccine against diphtheria, pertussis, tetanus, polio, *Haemophilus*, and hepatitis B for children. These combined vaccines have been shown to be well tolerated and safe. They have comparable rates of development of protective antibody levels compared to monovalent vaccines (95,96).

Various strategies have been examined to improve immunogenicity of HBV vaccines. One approach is to use more potent adjuvants (97,98). Another approach is to activate mucosal T cells through nasal vaccination (99). Other approaches include intradermal administration, coadministration with interleukin-2, and incorporation of pre-S1 and/or pre-S2 antigens (100–103).

## Diagnosis

The diagnosis of hepatitis B was revolutionized by the discovery of Australia antigen, now called HBsAg, by Blumberg in 1965 (104). During the ensuing decade, serologic assays for HBsAg and anti-HBs with increasing sensitivity and specificity were developed. In the 1970s, additional HBV antigens and antibodies were identified and serologic assays for their detection established. Advances in molecular biology techniques in the 1980s led to the development of hybridization assays for direct determination of virus replication and polymerase chain reaction (PCR) assays that permitted the detection of as little as ten molecules of HBV DNA per mL of serum. Diagnosis of HBV infection can also be made by the detection of HBsAg or HBcAg in liver tissues by immunohistochemical staining and of HBV DNA by Southern hybridization, in situ hybridization or PCR.

### SEROLOGIC DIAGNOSIS

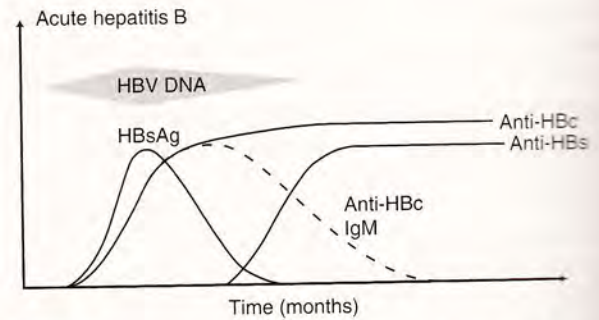
Serological markers during HBV infection are shown in Figures 29.1 and 29.2 and Table 29.4.

#### Hepatitis B surface antigen and hepatitis B surface antibody

HBsAg is the serologic hallmark of HBV infection. It can be detected by radioimmunoassays (RIAs) or enzyme immunoassays (EIAs). HBsAg appears in serum 1 to 10 weeks after an acute exposure to HBV, approximately 2 to 6 weeks before the onset of hepatitis symptoms or elevation of alanine aminotransferase (ALT) (105).

**TABLE 29.4. INTERPRETATION OF HEPATITIS B VIRUS (HBV) SEROLOGIC MARKERS**

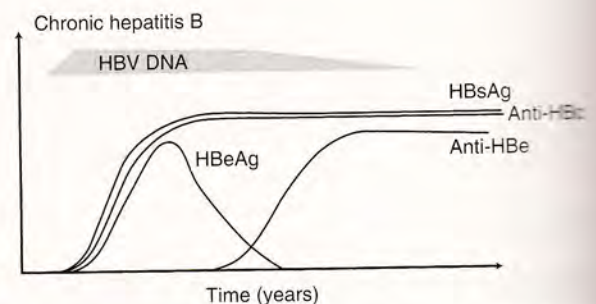
Hepatitis B surface antigen	HBV infection: acute or chronic
Hepatitis B e antigen	High levels of HBV replication and infectivity
Anti-HBe	Low levels of HBV replication and infectivity
Anti-HBc (IgM)	Recent HBV infection
Anti-HBc (IgG)	Recovered or chronic HBV infection
Anti-HBs	Immunity to HBV infection
Anti-HBc (IgG) + anti-HBs	Past HBV infection
Anti-HBc (IgG) + HBsAg	Chronic HBV infection



**Figure 29.1** Serologic markers during acute hepatitis B virus infection. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBc, hepatitis B core antibody; anti-HBs, hepatitis B surface antibody; IgM, immunoglobulin M.

In patients who subsequently recover, HBsAg usually becomes undetectable after 4 to 6 months. Persistence of HBsAg for more than 6 months implies chronic infection. The disappearance of HBsAg is followed by the appearance of anti-HBs. Although anti-HBs is produced early in the course of acute infection in individuals who subsequently recover, they are frequently not detectable until after a window period of several weeks to months when neither HBsAg nor anti-HBs can be detected (105) (Fig. 29.1). The appearance of anti-HBs marks the recovery from hepatitis B. In most patients, anti-HBs persist for life, therefore conferring long-term immunity. Anti-HBs is the only protective antibody induced by most of the currently available vaccines, which consist of recombinant HBsAg only.

HBV can be classified into eight genotypes (106,107) and four major serotypes (108). All HBV serotypes share one common antigenic determinant, “a,” which is a conformational epitope located in the HBsAg. There are two additional pairs of mutually exclusive subtypic determinants “d” or “y” and “w” or “r” constituting the four major serotypes—adr, ayr, adw, and ayw. Antibodies to the “a” determinant confer protection to all HBV serotypes (109). At least 50% of the anti-HBs



**Figure 29.2** Serologic markers during chronic hepatitis B virus infection. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBc, hepatitis B core antibody; anti-HBe, hepatitis B e antibody.

that develops after recovery from acute hepatitis B or immunization with hepatitis B vaccines are directed against the "a" determinant, therefore providing cross-protection against other serotypes of HBV.

Coexistence of HBsAg and anti-HBs has been reported in approximately 24% of HBsAg-positive individuals (110). In most instances, the antibodies are directed against one of the subtypic determinants and not the common "a" determinant and are unable to neutralize the circulating virions (111). These individuals should therefore be regarded as carriers.

Pre-S1 and pre-S2 antigens have been detected in patients infected with HBV. In general, the presence of these antigens correlates with the detection of HBV DNA and virus replication (112). During recovery from acute hepatitis B, antibodies to pre-S1 and pre-S2 antigens appear early (113), prior to the detection of anti-HBs.

### **Hepatitis B core antigen and antihepatitis B core antibody**

HBcAg is an intracellular antigen that is expressed in infected hepatocytes. It is not detectable in serum. Its antibody—anti-HBc—however, can be detected throughout the course of HBV infection. During acute HBV infection, anti-HBc is predominantly immunoglobulin (Ig) M class. IgM anti-HBc is the first antibody to be detected (Fig. 29.1). It usually appears within 1 month after the appearance of HBsAg, approximately 1 to 2 weeks before ALT begins to rise (105). It is the sole marker of HBV infection during the window period, that is, the time gap between the disappearance of HBsAg and the appearance of anti-HBs (114). During convalescence, the titer of IgM anti-HBc declines while the titer of IgG anti-HBc increases. Therefore, the detection of IgM anti-HBc is usually taken as an indication of acute HBV infection. However, in 20% of patients, IgM anti-HBc may remain detectable up to 2 years after the acute infection (115). In addition, low-titer IgM anti-HBc persists in most patients with chronic HBV infection. Therefore, the reliability of IgM anti-HBc in the differentiation of acute from chronic HBV infection depends on the cutoff level in the assay. Even in assays with high cutoff values, IgM anti-HBc can be detected in patients with chronic HBV infection during exacerbations (116). This may lead to misdiagnosis of acute hepatitis B in patients who are not previously known to have chronic HBV infection and overestimation of the rate of progression to chronicity. Recent studies in endemic areas demonstrated that many patients presenting with acute hepatitis who test positive for HBsAg have exacerbations of chronic hepatitis B and not acute hepatitis B, and that less than 1% of immunocompetent adult patients with genuine acute hepatitis B progress to chronic infection (12,117).

IgM anti-HBc titers have been reported to correlate with ALT and serum HBV DNA levels in patients with chronic hepatitis B especially during exacerbations (118). It has also been suggested that serial IgM anti-HBc titers may be useful in monitoring response to interferon (IFN) therapy (119).

IgG anti-HBc persists along with anti-HBs in patients who recover from acute hepatitis B and in association with HBsAg in those who progress to chronic HBV infection.

Isolated presence of anti-HBc in the absence of HBsAg and anti-HBs has been reported in 0.4% to 1.7% among blood donors in low prevalence areas (120,121) and in 10% to 20% of the population in endemic countries (15,122). Isolated detection of anti-HBc may occur during the window period of acute hepatitis B when the anti-HBc is predominantly IgM class, many years after recovery from acute hepatitis B when anti-HBs has fallen to undetectable levels, or after many years of chronic HBV infection when HBsAg titer has decreased below the level for detection. The clinical significance of isolated anti-HBc is complex. Although HBV DNA has been detected in the serum of individuals with isolated anti-HBc when tested by PCR assays, the frequency of detection varies from 0% to 20% (18,123,124). Transmission of HBV infection has been reported from blood and organ donors with isolated anti-HBc but the incidence ranged from 0.4% to 78% (18,47,48,125,126), the risk being highest when livers from anti-HBc-positive donors are transplanted into seronegative recipients. Several studies found that 50% to 70% of asymptomatic individuals with isolated anti-HBc have false-positive test results (15,16), the false-positive rate has decreased with improved anti-HBc assays. The evaluation of individuals with isolated anti-HBc should include repeat testing for anti-HBc, HBsAg, anti-HBs, and hepatitis B e antibody (anti-HBe). Individuals with evidence of chronic liver disease should be tested for HBV DNA to exclude low-level chronic HBV infection.

### **Hepatitis B e antigen and hepatitis B e antibody**

HBeAg is a secretory protein that is processed from the precore protein. It is generally considered to be a marker of HBV replication and infectivity. Its presence is usually associated with the detection of HBV DNA polymerase (127) and HBV DNA (128) in serum. Epidemiologic studies reported significantly higher rates of transmission of HBV infection from HBeAg-positive carrier mothers to their babies (129,130) and from HBeAg-positive patients to health care workers who sustain needle stick injuries (127).

During acute HBV infection, HBeAg appears shortly after the appearance of HBsAg. In patients who recover,

HBeAg to anti-HBe seroconversion precedes that of HBsAg to anti-HBs seroconversion (105) (Fig. 29.1). Anti-HBe may persist for many years after resolution of acute hepatitis B. In patients with chronic HBV infection, HBeAg may persist for years to decades (Fig. 29.2). During the HBeAg-positive phase, most patients have detectable HBV DNA in serum and active liver disease. In patients with perinatally acquired HBV infection, there may be an immune tolerant phase with normal ALT levels and minimal inflammation in the liver (131–133). Seroconversion from HBeAg to anti-HBe is usually associated with marked decrease in serum HBV DNA level and remission of liver disease (134–136). However, some anti-HBe-positive patients continue to have active liver disease and detectable HBV DNA in serum (128,137). This may be due to low levels of wild type HBV or the presence of precore HBV variants (138).

### TESTS FOR HEPATITIS B VIRUS DEOXYRIBONUCLEIC ACID IN SERUM

Assays for HBV DNA polymerase activity were developed in the 1970s to directly assess and quantify HBV replication (139). These assays are cumbersome and have been superseded by assays to detect HBV DNA. Serum HBV DNA levels can be quantified by molecular hybridization or signal amplification assays, which have sensitivity limits of  $10^5$  to  $10^6$  viral copies/mL. PCR assays are more sensitive and are capable of detecting less than  $10^2$  copies/mL (140). An arbitrary value of greater than  $10^5$  copies/mL has been chosen as a diagnostic criterion for chronic hepatitis B (141). However, there are problems with this definition. First, assays for HBV DNA quantification are not well standardized (Table 29.5) (142–146). In the last few years, there has been concerted effort to mandate standardization of all HBV DNA assays against WHO standards and to report results in international units (147,148), but this process of standardization has not been yet been implemented worldwide. Second, some patients with chronic hepatitis B have fluctuating HBV DNA levels that may at times fall below  $10^5$  copies/mL (149–151). Therefore,

a single HBV DNA level on a random occasion may not be accurate in assessing HBV replicative status and need for antiviral therapy in individual patients. Third, the threshold HBV DNA level that is associated with progressive liver disease is unknown.

Using hybridization assays, HBV DNA can be detected approximately 1 week after the appearance of HBsAg in patients with acute hepatitis B (152). In rare cases, HBV DNA can be detected before HBsAg. PCR assays can detect HBV DNA earlier, up to 2 to 3 weeks before the appearance of HBsAg. Recovery from acute hepatitis B is usually accompanied by the disappearance of HBV DNA in serum as determined by hybridization or bDNA assays. However, HBV DNA may remain detectable in serum for many years if tested by PCR assays (153).

In patients with chronic HBV infection, spontaneous or treatment induced HBeAg seroconversion is usually accompanied by the disappearance of HBV DNA in serum as determined by unamplified assays but HBV DNA frequently remains detectable by PCR assays except in patients who have HBsAg seroconversion (154). The major role of serum HBV DNA assays in patients with chronic HBV infection is to assess HBV replication and candidacy for antiviral therapy. Patients with high pretreatment serum HBV DNA levels are less likely to respond to IFN therapy but pretreatment serum HBV DNA levels seem to be less important in predicting response to nucleoside/nucleotide analogs (155,156). Tests for HBV DNA in serum are also important in assessing response to antiviral treatment.

Rarely, tests for HBV DNA in serum help to identify HBV as the etiology of liver disease in HBsAg-negative patients (157,158). This is especially important in patients with fulminant hepatitis B, who may have cleared HBsAg by the time they present (159). In patients with chronic liver disease due to occult HBV infection, most cases are due to low levels of HBV whereas a small percent may be related to HBV mutants that downregulate the production of HBsAg or produce aberrant HBsAg that cannot be detected in conventional serologic assays (160).

**TABLE 29.5. COMPARISON OF HEPATITIS B VIRUS (HBV) DNA QUANTIFICATION ASSAYS**

Assay (manufacturer)	Volume of sample	Sensitivity <sup>a</sup>		Linearity (copies/mL)
		pg/mL	Copies/mL	
Branched DNA (Bayer)	10 $\mu$ L	0.002	$2 \times 10^3$	$3 \times 10^3$ – $8 \times 10^8$
Hybrid capture (Digene)	30 $\mu$ L	0.5	$1.4 \times 10^5$	$2 \times 10^5$ – $1 \times 10^9$
	1 mL	0.02	$5 \times 10^3$	$5 \times 10^3$ – $3 \times 10^6$
PCR—Amplicor (Roche)	50 $\mu$ L	0.001	$4 \times 10^2$	$4 \times 10^2$ – $1 \times 10^7$
				Cobas: $10^2$ – $10^5$
				Taqman: $10^2$ – $10^{10}$
Molecular Beacons	10–50 $\mu$ L	—	<50	$50$ – $1 \times 10^9$

1 IU = approximately 5.6 copies/mL (depending on assay).

<sup>a</sup>One picogram of hepatitis B virus DNA = 283,000 copies (approximately  $3 \times 10^5$  viral genome equivalents).

**TABLE 29.6. DIAGNOSIS OF HEPATITIS B VIRUS (HBV) INFECTION**

	HBsAg	HBeAg	IgM anti-HBc	IgG anti-HBc	Anti-HBs	Anti-HBe	HBV DNA	Interpretation
Acute HBV infection	+	+	+	-	-	-	+++	Early phase
	-	-	+	-	-	+	+	Window phase
	+	+	-	+	+	-	±	Recovery phase
Chronic HBV infection	+	-	-	+	-	-	+++	HBeAg+ chronic hepatitis
	+	-	-	+	-	+	±	Inactive carrier state
	+	-	-	+	-	+	++	HBeAg- chronic hepatitis
	+	±	±	+	-	±	++	Exacerbations of chronic hepatitis

HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; IgM, immunoglobulin M; anti-HBc, hepatitis B core antibody; IgG, immunoglobulin G; anti-HBs, hepatitis B surface antibody; anti-HBe, hepatitis B e antibody.

Currently, most diagnostic laboratories use real-time PCR assays which have a wider range of linearity and are more accurate in quantification of HBV DNA. Some real-time PCR assays can also differentiate among various HBV genotypes and wild type versus HBV mutants such as precore mutation and antiviral-resistant mutations (161,162).

## DIAGNOSTIC ALGORITHM

Diagnosis of HBV infection is shown in Table 29.6 and Figures 29.1 and 29.2.

The diagnosis of acute hepatitis B is based on the detection of HBsAg and IgM anti-HBc. During the initial phase of infection, markers of HBV replication: HBeAg and HBV DNA are present. Recovery is accompanied by the disappearance of HBV DNA, HBeAg to anti-HBe seroconversion and subsequently HBsAg to anti-HBs seroconversion. Rarely, patients may have entered the window period at the time of presentation; IgM anti-HBc is the sole marker of acute HBV infection in these patients. This situation is more common in patients with fulminant hepatitis B where virus clearance tends to be more rapid.

Past HBV infection is diagnosed by the detection of anti-HBs and anti-HBc (IgG). Immunity to HBV infection after vaccination is indicated by the presence of anti-HBs only.

The diagnosis of chronic HBV infection is based on the detection of HBsAg and IgG anti-HBc. Additional tests for HBV replication: HBeAg and serum HBV DNA should be performed to determine if the patient should be considered for antiviral therapy. Additional tests for hepatitis C and hepatitis D should also be performed to rule out superinfection with other hepatitis virus(es).

## Clinical Manifestations

The spectrum of HBV infection varies from subclinical hepatitis, anicteric hepatitis, icteric hepatitis to fulminant hepatitis during the acute phase; and from inactive

carrier state to chronic hepatitis, cirrhosis and HCC during the chronic phase. The clinical manifestations and outcome of HBV infection depend on the age at infection, the level of HBV replication and the immune status of the host. Perinatal or childhood infection is usually associated with few or no symptoms but a high risk of chronicity whereas adult acquired infection is usually associated with symptomatic hepatitis but a low risk of chronicity. The consensus definition and diagnostic criteria for clinical terms relating to HBV infection adopted at a recent National Institutes of Health (NIH) workshop are summarized in Table 29.7 (141).

## ACUTE HEPATITIS B VIRUS INFECTION

Approximately 70% of patients have subclinical or anicteric hepatitis during acute HBV infection, only 30% have icteric hepatitis. Symptomatic hepatitis is rare in neonates and it occurs in approximately 10% of children less than 4 years old and in approximately 30% of adults (163).

### Symptoms and signs

The incubation period of acute HBV infection lasts 1 to 4 months. This period may be shorter in patients who have been exposed to a large inoculum (164). During the prodromal period, a serum sickness-like syndrome may develop. This is followed by insidious onset of constitutional symptoms including malaise, anorexia, nausea and occasionally vomiting, low-grade fever, myalgia and easy fatigability. Patients may have altered gustatory acuity and smell sensation. Some patients may experience intermittent mild to moderate right upper quadrant or midepigastic pain. In patients with icteric hepatitis, jaundice usually begins within 10 days after the onset of constitutional symptoms. Constitutional symptoms generally subside as jaundice develops. Clinical symptoms and jaundice usually disappear after 1 to 3 months but some patients may have persistent fatigue even after the ALT levels have returned to normal.

m  
cont

n

**TABLE 29.7. GLOSSARY OF CLINICAL TERMS USED IN HEPATITIS B VIRUS (HBV) INFECTION****DEFINITIONS****Chronic hepatitis B**

Chronic necroinflammatory disease of the liver caused by persistent infection with hepatitis B virus. Chronic hepatitis B can be subdivided into HBeAg positive and HBeAg negative chronic hepatitis B.

**Inactive HBsAg carrier state**

Persistent HBV infection of the liver without significant, ongoing necroinflammatory disease.

**Resolved hepatitis B**

Previous HBV infection without further virological, biochemical or histologic evidence of active virus infection or disease.

**Acute exacerbation or flare of hepatitis B**

Intermittent elevations of aminotransferase activity to more than ten times the upper limit of normal and more than twice the baseline value.

**Reactivation of hepatitis B**

Reappearance of active necroinflammatory disease of the liver in a person known to have the inactive HBsAg carrier state or resolved hepatitis B.

**HBeAg clearance**

Loss of HBeAg in a person who was previously HBeAg positive.

**HBeAg seroconversion**

Loss of HBeAg and detection of anti-HBe in a person who was previously HBeAg positive and anti-HBe negative, associated with decrease in serum HBV DNA to  $<10^5$  copies/mL.

**HBeAg reversion**

Reappearance of HBeAg in a person who was previously HBeAg negative, anti-HBe positive.

**DIAGNOSTIC CRITERIA****Chronic hepatitis B**

1. HBsAg+  $>6$  m
2. Serum HBV DNA  $>10^5$  IU/mL (may be lower for HBeAg negative patients)
3. Persistent or intermittent elevation in ALT/AST levels
4. Liver biopsy showing chronic hepatitis (necroinflammatory score  $\geq 4$ )<sup>a</sup>

**Inactive HBsAg carrier state**

1. HBsAg+  $>6$  m
2. HBeAg-, anti-HBe+
3. Serum HBV DNA  $<10^5$  IU/mL (usually  $<10^3$  IU/mL)
4. Persistently normal ALT/AST levels
5. Liver biopsy confirms absence of significant hepatitis (necroinflammatory score  $<4$ )<sup>a</sup>

**RESOLVED HEPATITIS B**

1. Previous known history of acute or chronic hepatitis B or the presence of anti-HBc  $\pm$  anti-HBs
2. HBsAg-
3. Undetectable serum HBV DNA<sup>b</sup>
4. Normal ALT levels

<sup>a</sup>Optional.

<sup>b</sup>Very low levels may be detectable using sensitive PCR assays.

HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBc, hepatitis B core; anti-HBs, hepatitis B surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Adapted from Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000—summary of a workshop. *Gastroenterology* 2001;120(7):1828–1853.

Physical examination can be unrevealing in many patients. The most common findings include low-grade temperature, clinical icterus, and soft mildly tender hepatomegaly. Splenomegaly may be found in approximately 5% to 15% of patients. Rarely palmar erythema or spider nevi can be detected. Mild lymph node enlargement may be present, but generalized lymphadenopathy is not a feature of acute hepatitis B.

Patients with fulminant hepatitis may present with features of hepatic encephalopathy—disturbance in sleep pattern, asterixis, mental confusion, disorientation, somnolence and coma, progressive decrease in liver span, and ascites.

**Laboratory findings**

Elevation of liver enzymes—alanine aminotransferase (ALT) and aspartate aminotransferase (AST)—is the

hallmark of acute hepatitis. Values of 1,000 to 2,000 IU/L are typically seen during the acute phase. Increase in liver enzymes may precede the onset of symptoms. The ALT levels are usually higher than the AST levels. In patients with icteric hepatitis, increase in bilirubin levels usually lags behind increase in ALT levels. Although the peak ALT level reflects the degree of hepatocellular injury, it has no correlation with prognosis. Prothrombin time, which is a measure of liver derived clotting factors II, VII, IX, and X, is the best indicator of prognosis in patients with acute hepatitis. Because of the short half-life of these clotting factors (6 hours for factor VII), prothrombin time reflects the instantaneous synthetic function of the liver and the mass of viable hepatocytes. Serum albumin level is not a good marker of liver function during acute hepatitis because of its long half-life (21 days). Mild leukopenia with relative lymphocytosis is a common finding. Although red cell survival is slightly shortened in acute hepatitis, hemoglobin and hematocrit are usually within normal limits. Rare hematologic findings include hemolytic anemia associated with glucose-6-phosphatase deficiency (165) and aplastic anemia (166). In patients who recover, ALT levels usually return to normal values after 1 to 4 months, followed by normalization in bilirubin levels. Persistent elevation of ALT levels for more than 6 months suggests chronic liver injury and persistent infection.

### Histologic findings

Liver biopsy is seldom indicated in acute hepatitis. Histologic changes of acute hepatitis include lobular disarray, acidophilic degeneration of hepatocytes, focal lobular necrosis, disruption of bile canaliculi with cholestasis, portal and parenchymal infiltration of inflammatory cells, as well as hypertrophy and hyperplasia of Kupffer cells and macrophages. Inflammatory infiltrates are predominantly lymphocytes and macrophages, with occasional eosinophils and neutrophils, but rarely plasma cells. In patients with severe hepatitis, hepatocyte necrosis is more extensive leading to bridging or linking up of necrotic areas in adjacent lobules. Resolution of hepatitis is signified by reduction of inflammatory infiltrates and parenchymal cell regeneration. In some cases of subfulminant hepatitis, liver biopsy may be indicated to assess the extent of liver necrosis and the need for a liver transplantation.

### Sequelae

The risk of chronicity is inversely proportional to the age at infection. **Less than 5% of immunocompetent adults with acute HBV infection progress to chronic infection, but up to 90% of those infected during infancy will develop chronic infection (5,10–12).** **Acute HBV infection is estimated to account for 35% to 70% of all virally related cases of fulminant hepatitis**

(167–169) but only 0.1% to 0.5% of acute hepatitis B runs a fulminant course (167,170). Mortality from fulminant hepatitis B is high, approaching 80%, unless liver transplantation can be performed. Contrary to transplantation for HBV-cirrhosis, reinfection after liver transplantation for fulminant hepatitis B is uncommon.

## CHRONIC HEPATITIS B INFECTION

### Symptoms and signs

In areas of low or intermediate prevalence, approximately 30% to 50% of patients with chronic HBV infection have a history of classical acute hepatitis that progressed to chronic infection (171,172). The remaining 50% to 70% of patients with chronic HBV infection in these areas and most of those in high prevalence areas (predominantly perinatal infection) have no prior history of acute hepatitis. **Many patients with chronic HBV infection are asymptomatic while others have nonspecific symptoms such as fatigue. Occasionally mild right upper quadrant or midepigastic pain may be present.** Patients with chronic HBV infection may experience exacerbations that may be asymptomatic **or mimic acute hepatitis with fatigue, anorexia, nausea and jaundice and in rare instances hepatic decompensation.**

**Physical examination may be unrevealing or there may be stigmata of chronic liver disease such as spider angioma and palmar erythema, and a mild hepatomegaly. In patients with cirrhosis, additional findings such as splenomegaly may be present. As the liver disease advances, hepatic decompensation may develop manifesting as variceal bleeding, ascites, peripheral edema, jaundice, and hepatic encephalopathy.**

### Laboratory findings

Laboratory tests can be entirely normal even in patients with well-compensated cirrhosis. Mild to moderate liver enzyme elevation may be the only biochemical abnormality in many patients with chronic hepatitis B. ALT levels may range from normal to fivefold elevated and are generally higher than AST levels, except in patients who have progressed to cirrhosis. Very high ALT levels, up to 1,000 IU/L, may be seen during exacerbations. Markers of impaired hepatic synthetic function may be observed during the exacerbations, especially in patients with underlying cirrhosis. In addition, increase in  $\alpha$ -fetoprotein (AFP) levels, up to 1,000 ng/mL, may be present (173). The AFP levels tend to parallel or follow the ALT levels. Progression to cirrhosis is suspected when platelet count is decreased, when there is hypoalbuminemia, hyperbilirubinemia and prolongation in prothrombin time, and when AST/ALT ratio is greater than 1.

## Histologic findings

Liver biopsy is useful in assessing the severity of liver damage, in predicting prognosis and in monitoring response to treatment. However, it must be recognized that liver histology can improve significantly in patients who have sustained response to antiviral therapy or spontaneous HBeAg seroconversion. Liver histology also can worsen rapidly in patients during exacerbations of hepatitis.

The predominant histologic findings include inflammatory cell infiltration in the portal tracts and periportal necrosis. The inflammatory infiltrate consists mainly of mononuclear cells. Periportal necrosis may be mild or severe leading to disruption of the limiting plate (piecemeal necrosis or interface hepatitis). As the liver damage progresses, fibrous tissue is deposited initially within the portal tracts, later extending into the centrilobular areas and adjacent portal tracts forming bridging fibrosis and eventually cirrhosis. In some patients, ground glass hepatocytes that stain positive for HBsAg can be found. Recent studies showed that these cells are found in association with retention of HBsAg (174). Traditionally, the histology of chronic hepatitis B is divided into chronic persistent hepatitis (CPH), chronic active hepatitis (CAH), and cirrhosis (175). CPH represents a milder form of liver injury with limitation of chronic inflammatory infiltrates to the portal tracts whereas CAH represents a more severe form of liver injury characterized by the presence of piecemeal necrosis. A third form of liver injury had been described—chronic lobular hepatitis (CLH) (176). CLH is characterized by spotty necrosis and inflammation within the lobules with minimal or mild portal inflammation. It is most often seen during exacerbations of chronic hepatitis B. CPH and CAH were thought to represent two dichotomous reactions to chronic HBV infection with different prognosis. The advent of antiviral therapy and the availability of serial liver biopsies before and after spontaneous or treatment induced HBeAg seroconversion revealed that patients may progress from CPH to CAH and vice versa suggesting that these two forms of liver injury may be seen during different phases of chronic HBV infection in the same patient. To provide more objective assessment of liver injury, several numerical scoring systems have been established to permit statistical comparisons of necroinflammatory activity and fibrosis (177–179). An international panel recommended that the histologic diagnosis include the etiology of hepatitis, the grade of necroinflammatory activity, and the stage (extent of fibrosis) of the liver disease (180).

Immunohistochemical staining reveals the presence of HBsAg in patients with chronic HBV infection. The distribution of HBsAg can be either membranous or cytoplasmic. In patients with high levels of HBV

replication, HBcAg can also be demonstrated. The distribution of HBcAg is usually nuclear but it has been observed that the distribution is shifted to the cytoplasm in patients with exacerbations or active liver disease (181).

## EXTRAHEPATIC MANIFESTATIONS

Extrahepatic manifestations have been reported in patients with both acute and chronic HBV infection. Extrahepatic manifestations are more commonly associated with acute hepatitis B than other forms of acute viral hepatitis and may be present in approximately 10% to 20% of patients with chronic HBV infection. They are believed to be mediated by circulating immune complexes, the formation of which is favored by high levels of HBV replication (182,183).

### Serum sickness

Acute hepatitis B is sometimes heralded by a serum sickness–like syndrome manifested as fever, skin rash, polyarthralgia, and arthritis. Skin and joint manifestations usually subside rapidly with the onset of jaundice.

### Polyarteritis nodosa

Approximately 10% to 50% of patients with polyarteritis nodosa (PAN) are found to be HBsAg positive. The decline in HBV infection over the past decade, especially in developed countries has also been associated with a decrease in frequency of HBV-related PAN (183,184). Immune complexes involving HBV antigens and antibodies are believed to trigger the vascular injury (185). Vasculitis may affect large, medium and small sized vessels in multiple organs including cardiovascular (pericarditis, hypertension, cardiac failure), renal (hematuria, proteinuria), gastrointestinal (abdominal pain, mesenteric vasculitis), musculoskeletal (arthralgia, arthritis), neurologic (mononeuritis, central nervous system involvement) and dermatologic (rashes) systems. The course is highly variable. Gastrointestinal involvement, especially perforation and bleeding, are the most severe manifestations and can be fatal (186). For many years, HBV-related PAN had been treated similar to non-virus-related PAN. Despite combination treatment with corticosteroid, immunosuppressive drugs and plasma exchange, mortality is high: 20% to 45% in 5 years and the outcome appears to be poorer than in non-virus-related PAN (184,187). The fact that HBV-related PAN is related to virus-mediated immune complexes suggests that therapy should be directed against HBV itself. Case reports and small case series have suggested a possible role of IFN therapy alone or in combination with plasma exchange (188–190), nucleoside analogs (191) or corticosteroids (192), as well as prednisone followed by lamivudine (193).



## Glomerulonephritis

HBV-related glomerulonephritis is more often found in children. Membranous glomerulonephritis is most common especially among children but membranoproliferative glomerulonephritis, mesangiocapillary and focal glomerulonephritis, minimal change disease and IgA nephropathy have also been reported (194). Immune complexes of hepatitis B surface, core, and e antigens and antibodies together with complement components have been demonstrated in glomerular basement membrane and mesangium (195). Liver disease tends to be mild in patients who present with HBV-related glomerulonephritis (196). Severity of the renal disease does not correlate with the severity of the liver disease or the level of HBV replication.

Approximately 30% to 60% of children with HBV-related membranous glomerulonephritis undergo spontaneous remission. Disease remission is especially evident after HBeAg seroconversion (197,198). A significant percent of adults (30%) may progress to renal failure and as many as 10% will require maintenance dialysis (199,200). Corticosteroids are usually ineffective for treatment of HBV-related glomerulonephritis and may potentiate HBV replication (201). IFN has been reported to induce remission of HBV-related renal disease in small clinical trials (202,203) but the response has been poor especially in Asians. In Western countries, response to IFN has been more favorable in adults, especially among patients with membranous glomerulonephritis (204). Improvement of renal disease has also been reported with lamivudine (205). Recent reports suggest that the incidence of HBV-related glomerulonephritis in children has been decreasing since the implementation of the HBV vaccination programs (206,207).

## Essential mixed cryoglobulinemia

Mixed cryoglobulinemia is a systemic disease involving mainly small vessels presenting as glomerulonephritis, arthritis and purpura. HBsAg, anti-HBs and HBV-like particles have been demonstrated in cryoprecipitates (208) but recent studies questioned the association between chronic HBV infection and essential mixed cryoglobulinemia.

## Papular acrodermatitis (Gianotti-Crosti disease)

Papular acrodermatitis is found to be strongly associated with HBs antigenemia in children particularly among those under the age of 4 (209,210). Circulating HBsAg and anti-HBs immune complexes are thought to play a role in the pathogenesis (209). It manifests as symmetrical, erythematous, maculopapular, nonitchy eruptions over the face, buttocks, limbs

and occasionally the trunk lasting for 15 to 20 days. Mucous membranes are spared. Lymphadenopathy particularly in the axillary and inguinal regions is common. Evidence of acute hepatitis may coincide with the onset of the skin eruption or, more commonly, begins as the dermatitis starts to wane.

## Aplastic anemia

Isolated cases of severe aplastic anemia occurring in the early phase of acute hepatitis have been reported (166). However, a recent study suggested that most hepatitis associated aplastic anemia are not related to hepatitis virus but mediated by immunopathologic mechanisms (211).

## SPECIAL PATIENT GROUPS



### Pediatric patients

HBV infection remains the most important cause of chronic hepatitis in pediatric patients. The clinical manifestation of chronic HBV infection in children is dependent on the age at infection. Perinatal HBV infection results in a high rate (90%) of chronic infection and a prolonged replicative phase. Children with perinatally acquired HBV infection are usually asymptomatic with normal ALT values despite high serum HBV DNA levels.

Acute HBV infection has been estimated to account for 10% to 25% of all cases of childhood acute hepatitis (212,213). Extrahepatic manifestations including arthralgia, arthritis, skin rash and Gianotti's papular acrodermatitis are common and have been reported in 25% of patients (213).

Approximately 15% to 30% of children with chronic HBV infection, who were infected during early childhood are symptomatic with elevated ALT levels and have chronic hepatitis on liver biopsies (214,215). These children have higher rates of spontaneous HBsAg and HBeAg seroconversion: approximately 2% and 15% to 20% per year, respectively (215) compared to children who were infected perinatally. Loss of HBsAg is uncommon (216–219).

Children with chronic HBV infection are more likely than adults to develop HBV-related glomerulonephritis. Progression of disease activity over time can be seen in approximately 50% of children (216,217), cirrhosis is uncommon but has been demonstrated in approximately 3.5% of children with chronic HBV infection at the time of presentation (214,220). Cirrhosis appears to be more frequent in boys and in those with a history of acute hepatitis (220). HCC has also been reported among children with chronic HBV infection (216,221). It is more common among Asian children, and in children with cirrhosis or a family history of HCC. Therefore, regular follow-up is important even in asymptomatic carrier children in these settings.

HBV related fulminant hepatitis is extremely rare in children. Most reported cases occurred in infants born to HBeAg-negative mothers (222,223). In a case series in Taiwan, 65% of 17 cases of childhood fulminant hepatitis were caused by HBV infection (224).

### Immunocompromised patients

The clinical manifestations and natural course of chronic HBV infection in immunocompromised patients may be different from that in immunocompetent patients because of enhanced HBV replication and weak immune response.

Immunosuppressive therapy can increase HBV replication directly by stimulating the glucocorticoid responsive element in the enhancer region of the HBV genome (225) or indirectly by diminishing immune clearance. Abrupt withdrawal of immunosuppressive therapy as in cyclical chemotherapy or rapid tapering of steroid treatment has been reported to be associated with exacerbations of liver disease in HBsAg carriers as well as in immune individuals (226). These exacerbations are believed to be due to massive lysis of infected hepatocytes as the immune system recovers. Although most of the exacerbations are asymptomatic, fatal hepatic decompensation has been reported (227).

Patients with chronic renal failure on hemodialysis have an increased risk of HBV infection (228,229). Dialysis patients are usually HBeAg and serum HBV DNA positive but have no symptoms, normal ALT levels and minimal liver damage on liver biopsies (230). The clinical course of postrenal transplantation is, however, very different, with exacerbations, rapid progression to cirrhosis and an increased risk of HCC and death from liver failure (231–235). Recent reports showed that preemptive treatment with lamivudine decreased the risk of reactivation of hepatitis B post transplantation (236,237). In addition, lamivudine has been reported to be effective in treating hepatitis flares and hepatic decompensation due to reactivation of hepatitis B after renal transplantation (236,238). The American Society of Nephrology recommended that HBsAg-positive renal allograft recipients should receive lamivudine beginning at the time of transplantation and continuing for at least 18 to 24 months (239).

Patients with HIV infection have a high prevalence of HBV infection. This is probably related to the similarities in the mode of transmission of HBV and HIV. Patients who are coinfecting with HBV and HIV tend to have higher serum HBV DNA levels, lower ALT levels, lower rate of spontaneous as well as treatment related HBeAg seroconversion, and higher risk of cirrhosis (240–243). Reactivation of HBV replication has been described in association with HIV infection (244) and may lead to acceleration of liver disease progression (245). The response to HBV vaccination in HIV infected

patients is also impaired (246). In contrast, HBV coinfection does not appear to have any significant effect on the rate of progression of HIV disease (242,247). More recently, severe cases of HBV disease exacerbation and deaths due to liver failure are being increasingly reported in patients receiving highly active antiretroviral therapy (HAART). The exacerbations are felt to be related to “immune reconstitution” with subsequent immune-mediated injury directed against infected hepatocytes (245,248). Several studies have shown that patients coinfecting with HIV and HBV have increased risk of liver-related mortality compared to those with HIV or HBV mono-infection (249,250).

## Natural History

The natural course of HBV infection is determined by the interplay between the virus: HBV replication, HBV genotype and viral variants; host: Age, gender, race/ethnicity, genetic make-up, and immune response; and environment: Alcohol, concomitant infection with other viruses—hepatitis C, hepatitis D, human immunodeficiency virus (HCV, HDV, HIV), and carcinogens such as aflatoxin.

### PROGRESSION FROM ACUTE TO CHRONIC HEPATITIS B VIRUS INFECTION

The overall rate of progression from acute to chronic HBV infection has been estimated to be 5% to 10%. The risk is inversely proportional to the age at infection: 90% for perinatal infection, 20% for childhood infection, and less than 5% for adult infection (5,10–12). Careful analyses of patients presenting with “acute hepatitis B” found that the risk of progression to chronic HBV infection among immunocompetent adults was less than 1% after exclusion of patients who had acute exacerbations of chronic HBV infection.

### HEPATITIS B VIRUS INFECTION IS A LIFE-LONG INFECTION

The advent of sensitive molecular virology assays has revolutionized the concept of viral clearance and recovery from HBV infection. Many studies found that HBV DNA and vigorous immune response to HBV antigens can be detected more than 10 years after recovery from acute HBV infection—HBsAg to anti-HBs seroconversion (251,252). These findings indicate that HBV persists but is contained by the host immune response. This accounts for reports of chemotherapy induced reactivation of HBV replication in patients with “recovered” HBV infection (226).

The likelihood of spontaneous viral clearance in patients with chronic HBV infection is very low because

of the presence of extrahepatic reservoirs of HBV, integration of HBV DNA into the host genome, and the presence of an intracellular conversion pathway whereby newly replicated HBV DNA re-enters the hepatocyte nuclei and is used to amplify covalently closed circular HBV DNA (cccDNA) (253). This intracellular pathway enables the establishment of a pool of transcriptional templates in the hepatocyte without the need for multiple rounds of reinfection. Therefore, spontaneous viral clearance is unlikely to occur once chronic HBV infection is established.

### e CLINICAL COURSE OF CHRONIC HEPATITIS B VIRUS INFECTION

The natural course of chronic HBV infection is characterized by fluctuations in level of HBV replication and activity of liver disease. The clinical course of chronic HBV infection can be considered as comprising **four** phases (Fig. 29.3) although not all patients go through every phase.

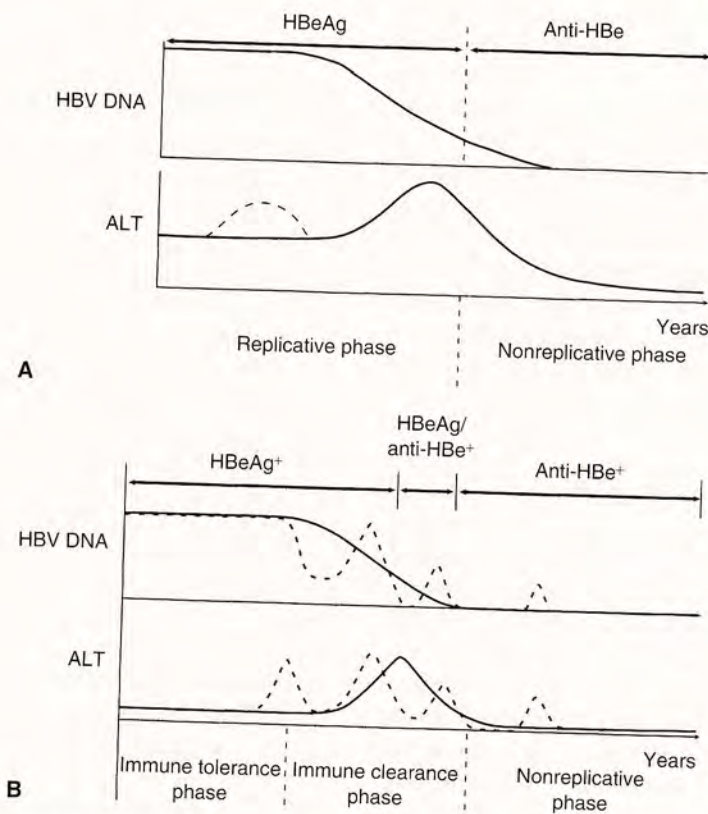
### f Immune-tolerant phase

In patients with perinatally acquired HBV infection, the initial phase is characterized by high levels of HBV replication: Presence of HBeAg and high levels of HBV DNA in serum ( $10^6$ – $10^{10}$  IU/mL), normal ALT

and minimal changes on liver biopsy (131,133,254). A mild degree of liver injury despite high levels of HBV replication is believed to be due to immune tolerance to HBV. The exact mechanism(s) for immune tolerance is unknown. Experiments in mice suggest that transplacental transfer of maternal HBeAg may induce a specific unresponsiveness of helper T cells to HBeAg (255). Because HBeAg and HBcAg are cross-reactive at the T-cell level, deletion of T-helper cell response to HBeAg results in ineffective cytotoxic T-cell response to HBcAg (256).

During the immune tolerance phase, which lasts 1 to 4 decades, there is a very low rate of spontaneous HBeAg clearance. The cumulative rate of spontaneous HBeAg clearance is estimated to be approximately 2% during the first 3 years (133,257) and only 15% after 20 years of infection (258). Persistence of high levels of viremia in adolescents and young adults accounts for the high frequency of maternal–infant transmission of HBV in Asia. The lack of assistance from immune-mediated viral clearance also contributes to a low rate of treatment-related HBeAg seroconversion.

A study from Taiwan followed 240 adult patients (54% men, mean age 28 years) who presented during this phase and found that only 5% progressed to cirrhosis and none to HCC during a mean follow-up of 10.5 years (259). The risk of cirrhosis was higher in



■ **Figure 29.3** Natural history of chronic hepatitis B virus (HBV) infection. **A:** Adult acquired HBV infection, **(B)** perinatally acquired HBV infection. HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody; HBV, hepatitis B virus; ALT, alanine aminotransferase.

patients who had HBeAg seroconversion at an older age and those who had relapse of hepatitis after HBeAg seroconversion. These findings indicate that presence of HBeAg at presentation is not invariably associated with high risks of cirrhosis and HCC, rather the risk of adverse clinical outcome is related to a long duration of high levels of HBV replication and active hepatitis.

In patients with childhood or adult acquired HBV infection, the “immune-tolerant” phase is short-lived or absent.

### **g** Immune clearance phase/hepatitis B e antigen–positive chronic hepatitis

This phase is characterized by the presence of HBeAg, high levels of serum HBV DNA and active liver disease (elevated ALT and necroinflammation on liver biopsy). In patients with perinatally acquired HBV infection, transition from the immune tolerant to the immune clearance phase usually occurs during the second to fourth decades of life. Most patients with childhood or adult acquired HBV infection are already in the immune clearance phase at presentation.

During this phase, spontaneous HBeAg clearance occurs at an annual rate of 10% to 20% (258–260) (Fig. 29.3). HBeAg seroconversion is frequently but not always accompanied by biochemical exacerbations (215,258,261). These exacerbations are believed to be due to a sudden increase in immune-mediated lysis of infected hepatocytes, and are often preceded by an increase in serum HBV DNA level (262) and a change in distribution of HBcAg from nuclear to cytoplasmic localization in the hepatocytes (181).

Most exacerbations are asymptomatic but some are accompanied by symptoms of acute hepatitis. Occasionally, IgM anti-HBc may be detected leading to misdiagnosis of acute hepatitis B in previously unrecognized carriers (117). Exacerbations may be associated with increase in  $\alpha$ -fetoprotein levels (173,260). In approximately 2.5% of patients (especially those with preexisting cirrhosis), exacerbations may result in hepatic decompensation and rarely death from hepatic failure (263).

Not all exacerbations lead to HBeAg seroconversion (258,264,265). Some patients have suboptimal immune response and abortive immune clearance. These patients may develop recurrent exacerbations with intermittently undetectable serum HBV DNA with or without transient loss of HBeAg. Repeated episodes of necroinflammation may increase the risk of cirrhosis and HCC. Exacerbations are more commonly observed in men than in women (265) and may account for a higher incidence of HBV-related cirrhosis and HCC among men.

An important outcome of the “immune clearance” phase is HBeAg to anti-HBe seroconversion. Factors

associated with higher rates of spontaneous HBeAg seroconversion include older age, higher ALT levels and more recently HBV genotype B (258,260,266–270). High ALT level is believed to be a reflection of vigorous host immune response accounting for its strong correlation with spontaneous as well as treatment-related HBeAg seroconversion. Studies from Asian countries where genotypes B and C predominate showed that HBV genotype B is associated with a lower prevalence of HBeAg, HBeAg seroconversion at an earlier age, and more sustained virologic and biochemical remission after HBeAg seroconversion (266,270).

### **h** Inactive carrier phase

This phase is characterized by absence of HBeAg, presence of anti-HBe, persistently normal ALT levels, and low or undetectable serum HBV DNA (usually  $<10^5$  IU/mL) (141). Liver biopsy generally shows mild hepatitis and minimal fibrosis but inactive cirrhosis may be observed in patients who had accrued substantial liver injury during the preceding “immune clearance” phase.

The inactive carrier phase may persist indefinitely, in which case the prognosis is generally favorable especially if this phase is reached early. This is supported by the finding of comparable survival between HBsAg-positive blood donors (almost all were HBeAg negative and had normal ALT at baseline) and uninfected controls over a 30-year period (271).

Some patients in the inactive carrier phase eventually clear HBsAg. The annual rate of HBsAg clearance has been estimated to be 0.5% to 2% (267,272). Despite HBsAg clearance, some patients have residual liver disease and some may develop HCC, the risk of HCC is higher in those who have progressed to cirrhosis prior to HBsAg clearance (273–275).

Some inactive carriers have reactivation of HBV replication later in life. Reactivation may occur spontaneously or as a result of immunosuppression, and may be due to wild type HBV or HBV variants that abolish or downregulate HBeAg production. In one study of 283 Chinese patients followed for a median of 8.6 years after spontaneous HBeAg seroconversion, 67% had sustained remission, 4% had HBeAg reversion and 24% had HBeAg-negative chronic hepatitis (276). Cirrhosis developed in 8% and HCC in 2%, the risk being higher in those who had active hepatitis after HBeAg seroconversion.

### **i** Reactivation of hepatitis B virus replication/hepatitis B e antigen–negative chronic hepatitis

This phase is characterized by absence of HBeAg, presence of anti-HBe, detectable serum HBV DNA, elevated ALT, and chronic inflammation  $\pm$  fibrosis on biopsy.

(Fig. 29.3) (141,277). Patients in this phase are usually older and have more advanced liver disease because this represents a later phase in the course of chronic HBV infection. Serum HBV DNA levels are lower than in HBeAg-positive patients but may reach  $10^{8-9}$  IU/mL.

The hallmark of this phase is its fluctuating course. In one study where patients were monitored at monthly intervals, 36% had persistently elevated ALT, 20% had fluctuating abnormal ALT, and 44% had fluctuating ALT with intermittently normal values (278). Several investigators have attempted to define cutoff HBV DNA levels that would differentiate patients with HBeAg-negative chronic hepatitis from inactive carriers; because of the fluctuating course, serial testing is more reliable than any single HBV DNA level at a random time point (213,215,279).

HBeAg-negative chronic hepatitis was originally reported in Mediterranean countries. It has now been reported in all parts of the world (280). The geographic variations in prevalence of HBeAg-negative chronic hepatitis are related to the predominant HBV genotype in that region. Recent studies in Europe, Asia and the United States have all reported an increased prevalence of HBeAg-negative and a decreased prevalence of HBeAg-positive chronic hepatitis (281,282). This may be related to increased awareness, decrease in new HBV infections due to vaccination, and progression of the existing pool of HBV carriers to later stages of chronic HBV infection.

### LATENT/OCCULT HEPATITIS B VIRUS INFECTION

Occlude HBV infection is defined as the detection of HBV DNA in persons who are HBsAg negative (283). HBV DNA is more often detected in liver than in serum. It has been proposed that diagnosis of occult HBV infection be made only when HBV DNA can be detected using at least two sets of primers from different regions of the HBV genome in duplicate assays.

Prevalence of occult HBV infection is higher in countries that are endemic for HBV and in individuals with serologic markers of HBV exposure. (283). HBV-DNA detection rate is highest in subjects who are anti-HBc positive/anti-HBs negative; some of these individuals probably have low-level HBV infection with subdetectable HBsAg. The HBV-DNA detection rate is intermediate in subjects who are positive for both anti-HBc and anti-HBs. These individuals may have recovered from previous infection. The HBV-DNA detection rate is lowest in seronegative subjects. These individuals have recovered from previous HBV infection but lost all serologic markers. Rarely, they may be infected with HBV mutants that do not express HBV serologic markers.

Occlude HBV infection is more common among patients with cirrhosis or HCC (284). Many of these patients probably had chronic HBV infection for decades leading to liver damage but HBsAg is no longer detectable when cirrhosis or HCC is diagnosed. Low levels of HBV may also be a cofactor of liver disease in patients with chronic HCV infection, nonalcoholic fatty liver disease,  $\alpha_1$ -antitrypsin deficiency and other causes of chronic liver disease. Whether occult HBV infection alone can cause cirrhosis or HCC is unclear.

### SEQUELAE OF CHRONIC HEPATITIS B VIRUS INFECTION

The sequelae of chronic HBV infection vary from inactive carrier state to chronic hepatitis, cirrhosis, hepatic decompensation, HCC and death. The clinical outcome of patients with chronic HBV infection depends on the severity of liver damage prior to sustained HBeAg seroconversion and the durability of the inactive carrier phase.

Annual rate of progression from chronic hepatitis to cirrhosis has been estimated to be 2% to 6% for HBeAg-positive and 8% to 9% for HBeAg-negative patients (171,267,285–287), the higher rate in HBeAg-negative patients is related to older age and more advanced liver disease at presentation. Factors that have been reported to be associated with increased rate of progression to cirrhosis include: Host (older age, men), virus (persistent high levels of HBV replication, HBV genotype [C > B], coinfection with HCV, HDV, and HIV), and environment (alcohol and more recently obesity) (267,285,287–294). One study from Taiwan found that the 10-year cumulative probability of cirrhosis among chronic hepatitis B patients with HCV, HDV, and no superinfection was 48%, 21%, and 9%, respectively (290). Several studies showed that patients who had HBeAg reversion had increased risk of cirrhosis compared to those who had sustained HBeAg seroconversion (267,276). One study reported that the adjusted relative risk of cirrhosis for patients with serum HBV DNA greater than  $10^4$  and greater than  $10^6$  copies/mL was 2.3 and 9.3 compared to carriers with serum HBV DNA less than  $10^4$  copies/mL (294). These data suggest that persistent high levels of HBV replication (with accompanying hepatitis) increase the risk of cirrhosis. Studies in Asia found that genotype C is associated with a more rapid rate of progression to cirrhosis than genotype B (291–293) possibly related to a longer duration of high levels of HBV replication and more active hepatitis.

Annual rate of progression from compensated cirrhosis to hepatic decompensation has been estimated to be 4% to 6% (287,295,296). Survival after the development of compensated cirrhosis is favorable initially (85% at 5 years) but decreases dramatically after the

onset of decompensation to 55% to 70% at 1 year and 14% to 35% at 5 years (276,295,297–300). The lifetime risk of a liver-related death was estimated to be 40% to 50% for men and 15% for women among Chinese patients with chronic HBV infection (301). Several studies revealed that persistent high level HBV replication is associated with increased mortality in patients with cirrhosis, while biochemical remission, clearance of HBeAg or HBV DNA from serum after the diagnosis of cirrhosis were associated with significantly higher rate of survival. (298,299).

Annual rate of HCC development has been estimated to be 0.5% to 1.0% for noncirrhotic carriers and 2.5% to 3% for patients with cirrhosis (171,285,295–297,300,302,303). Risk factors for HCC include host (older age, male gender, being Asian, and having first-degree relatives with HCC), virus (high levels of HBV replication, HBV genotype [C > B], core promoter mutations and coinfection with HCV), and environment (alcohol, aflatoxin, and more recently smoking, obesity, and diabetes) (291,292,295–297,300,301,303–310). It is important to note that although HCC is more common among patients with cirrhosis, 30% to 50% of HCC associated with HBV occurs in the absence of cirrhosis.

Recent studies found an association between HBV replication and the risk of HCC. In one study of 11,893 Taiwanese men aged 30 to 65 years, followed for a mean of 8.5 years, the adjusted relative risk of HCC was six- to sevenfold higher among HBsAg men who were HBeAg positive at entry than those who were HBsAg positive, HBeAg negative (306). Another study from Taiwan found that the risk of HCC increased with increasing baseline serum HBV DNA levels (307). These findings were confirmed by studies in Senegal and Mainland China (311). Unfortunately, none of these studies monitored serum HBV DNA and ALT levels over time. It is likely that the duration of high levels of HBV replication as well as the duration and severity of hepatitis activity are more important than a single high HBV DNA level on a random occasion in predicting the risk of HCC in individual carriers.

Several studies from Asia including the above study from Taiwan reported that genotype C is associated with increased risk of HCC compared to genotype B (291,292,307). This may be related to a longer duration of high levels of HBV replication and active hepatitis and a higher frequency of core promoter mutations. Core promoter mutations have been shown in some studies to be associated with increased risk of HCC and to precede HCC diagnosis (308,309,312). Core promoter mutations have been found to be associated with more active hepatitis and the most common mutations (A<sub>1762</sub>T, G<sub>1764</sub>A) result in corresponding changes in the overlapping X gene. In vitro studies

found that the HBx protein is a potent transactivator and may activate host genes including oncogenes (313).

## COINFECTION OF HEPATITIS B VIRUS AND HEPATITIS C VIRUS

Acute coinfection of HBV and HCV has been reported in transfused patients as well as in intravenous drug users (314,315). Coinfection with HCV may delay the onset and shorten the duration of HBs antigenemia as well as lower the peak ALT levels compared with acute HBV infection alone (314). These findings suggest that HCV coinfection may interfere with the replication of HBV leading to attenuation of liver damage. However, acute coinfection of HCV and HBV has been reported to increase the risk of fulminant hepatic failure (316). HCV superinfection in HBsAg carriers typically manifests as acute icteric hepatitis and is associated with a high risk of hepatic decompensation and death from hepatic failure (290). HCV superinfection has also been reported to decrease HBV DNA levels and to be associated with earlier HBsAg clearance (317–320). Most patients coinfecting with HCV and HBV have detectable serum HCV RNA but not HBeAg or HBV DNA indicating that HCV infection is dominant. Nevertheless, liver disease is usually more severe and the risks of cirrhosis and HCC are higher compared to patients infected with either virus alone (321,322).

## Hepatitis B Virus and Hepatocellular Carcinoma

Worldwide, HCC is the third most common cause of cancer deaths in men and the fifth most common cause of cancer deaths in women accounting for approximately 500,000 deaths each year (323). The vast majority (85%) of HCC is concentrated in eastern and southeastern Asia and sub-Saharan Africa, where HBV infection is endemic. Several lines of evidence support an etiologic association between HBV infection and HCC.

### EPIDEMIOLOGIC ASSOCIATION

There is a close correlation between the geographic distribution of HBsAg carriers and the occurrence of HCC (325–327). A high proportion of patients with HCC have chronic HBV infection, and HBV infection precedes HCC. The strongest evidence for an etiologic association between chronic HBV infection and the development of HCC is derived from a study of 11,893 Taiwanese men who were followed for a mean of 8.5 years (301). The incidence of HCC was 495/100,000

per year for HBsAg positive and 5/100,000 per year for HBsAg-negative men, with a relative risk of 98. The incidence of HCC/100,000 per year among HBsAg-negative men was 10, 5, and 0 for individuals who were anti-HBc alone positive, anti-HBc and anti-HBs positive, and seronegative, respectively.

## ANIMAL MODELS

Chronic infection with other hepadnaviruses has also been shown to be associated with the development of HCC. In one study, virtually 100% of the woodchucks chronically infected with woodchuck hepatitis virus (WHV) developed HCC 2 to 4 years after the onset of infection (328). These woodchucks were bred and raised in captivity and were fed strictly regulated food free of aflatoxin and other carcinogens. In the same study, only 6.5% of woodchucks had recovered from transient WHV infection and none of the uninfected woodchucks developed HCC. These data suggest that chronic infection with hepadnavirus alone is sufficient to cause HCC.

## PHYSICAL PRESENCE

The physical presence of HBV in HCC has been demonstrated by the finding of integrated HBV DNA in neoplastic liver tissues from most of the HBsAg-positive patients as well as some HBsAg-negative patients (329,330). In most instances, HBV DNA is present as a discrete high-molecular-weight band suggesting clonal expansion of hepatocytes that contain integrated HBV DNA. It should be noted that integrated HBV DNA can also be detected in adjacent non-neoplastic liver tissues as well as in liver tissues of patients with HBV-related chronic hepatitis or cirrhosis (330,331).

## PREVENTION OF HEPATOCELLULAR CARCINOMA THROUGH VACCINATION AND ANTIVIRAL THERAPY

Recent studies demonstrating that HBV-related HCC can be prevented through HBV vaccination and antiviral therapy provided further support of the etiologic association. Studies from Taiwan reported a significant decrease in the incidence of childhood HCCs that accompanied the decline in prevalence of chronic HBV infection among Taiwanese children after the implementation of universal vaccination of newborns (77).

Several follow-up studies of chronic hepatitis B patients who received IFN or lamivudine therapy observed a decrease in incidence of HCC among patients with long-term virologic response. The best evidence that antiviral treatment can prevent HCC was provided by a prospective double blind placebo-controlled trial of lamivudine treatment in patients

with high levels of HBV replication and bridging fibrosis or cirrhosis. After a median duration of 32 months, a significantly lower percent of treated patients had HCC (332). The efficacy of antiviral therapy in preventing HCC was also demonstrated in woodchucks that received lamivudine, clevudine and entecavir for chronic WHV infection (333,334).

## MECHANISMS OF HEPATOCARCINOGENESIS

Chronic HBV infection can induce HCC directly by activating cellular oncogenes or by inactivating tumor suppressor genes, or indirectly through chronic liver injury, inflammation, and regeneration (335).

To date there is no evidence that HBV DNA is directly oncogenic. Transfection of HBV DNA has not been demonstrated to induce malignant transformation of cultured cells. Analyses of integrated HBV DNA in neoplastic tissues from patients with HCC revealed varying portions of the HBV genome with deletions and duplications (335,336). An intact region of the HBV genome that is universally incorporated has not been identified. The long latent interval between the onset of HBV infection and the development of HCC also make it unlikely that HBV is an oncogenic virus.

Integration of HBV DNA may activate cellular proto-oncogenes or suppress growth-regulating genes. Recent studies found that HBV DNA integration frequently targets genes that regulate key cellular pathways such as calcium homeostasis, mean arterial pressure (MAP) kinase-dependent signaling pathways, and the telomerase gene (335,337).

Integration of HBV DNA can also induce carcinogenesis via transactivation. The HBx protein has been shown to be a potent and promiscuous transactivator of viral as well as cellular enhancer and promoter (313,338). Integration of the intact or truncated versions of the X gene is found in most HCCs. These sequences retain their transactivating capacity and may activate cellular oncogenes (339). It has also been reported that HBx protein binds the tumor suppressor gene p53 leading to decreased transcription and reduced cellular growth inhibition (340,341). Moreover, HBx protein has been shown to regulate proteasome function and to have an effect on calcium homeostasis and mitochondrial function (335). Finally, mutations in amino acids 130 and 131 of the X protein which corresponds to the most common core promoter mutations have been found in many patients with HCC and to precede HCC development (312). Another viral protein that may play a role in hepatocarcinogenesis is the 3'-truncated preS/S sequence which is frequently found in HCCs (342). More recently, a spliced HBV transcript and its encoded protein (HBSP) has also

t

been incriminated as playing a role in HCC development through its effect on HBV replication and liver fibrosis (343).

Integration of HBV DNA can also cause cancers indirectly via chromosomal deletions or translocations.

An alternate path by which chronic HBV infection leads to HCC is through induction of liver injury. Chronic liver cell injury initiates a cascade of events characterized by increased rates of cellular DNA synthesis and impaired cellular repair thereby setting the scene for acquired mutations. Mutations may be promoted by the release of inflammatory cytokines and exposure to environmental carcinogens. During regeneration, transformed cells that have a growth advantage are selected resulting in clonal expansion and eventually tumor formation. Experimental evidence for this hypothesis is derived from observations that transgenic mice which overexpress HBV large S protein develop severe hepatocellular injury followed by regenerative hyperplasia, transcriptional dysregulation, aneuploidy and finally neoplasia (174). Clinical studies support the conclusion that long durations of high levels of HBV replication and active hepatitis increase the risk of HCC.

## Hepatitis B Virus Genotypes and Variants

The HBV genome consists of four partially overlapping open reading frames: The *pre-S/S* gene that codes for the envelope proteins, the *pre-C/C* gene that codes for the e antigen and core protein, the *P* gene that codes for the DNA polymerase and reverse transcriptase, and the *X* gene that codes for a protein of unclear significance. Although HBV is a DNA virus, it is prone to mutations with a rate of nucleotide substitutions estimated at  $1 \times 10^{-5}$  to  $3 \times 10^{-5}$ /site per year (344). This is related to the reverse transcription of an RNA intermediate during the replication cycle of HBV. Mutations occur at random along the HBV genome but the overlapping open reading frames limit the number and location of viable mutations. Mutations that confer a survival advantage to the virus by enhancing replication or evading immune response tend to be selected.

### HEPATITIS B VIRUS GENOTYPES

HBV can be classified into eight genotypes designated A-H based on an intergroup divergence of 8% or more in the complete nucleotide sequence (106,107,345). The geographic distribution of HBV genotypes is summarized in Table 29.8. There is an association between HBV genotypes and precore and core promoter variants. The most common precore variant (G<sub>1896</sub>A) is predominantly found in patients with HBV genotypes B, C, and D and rarely in patients with genotype A

**TABLE 29.8. GEOGRAPHIC DISTRIBUTION OF HEPATITIS B VIRUS (HBV) GENOTYPES**

HBV genotypes	Distribution areas
A	North-west Europe, North America, Central Africa
B	China, Japan, Indonesia, Vietnam
C	China, Japan, East Asia, Korea, Vietnam, Polynesia
D	Mediterranean basin, Middle East, India
E	Africa
F	American natives, Polynesia
G	United States, France
H	Mexico, Latin America

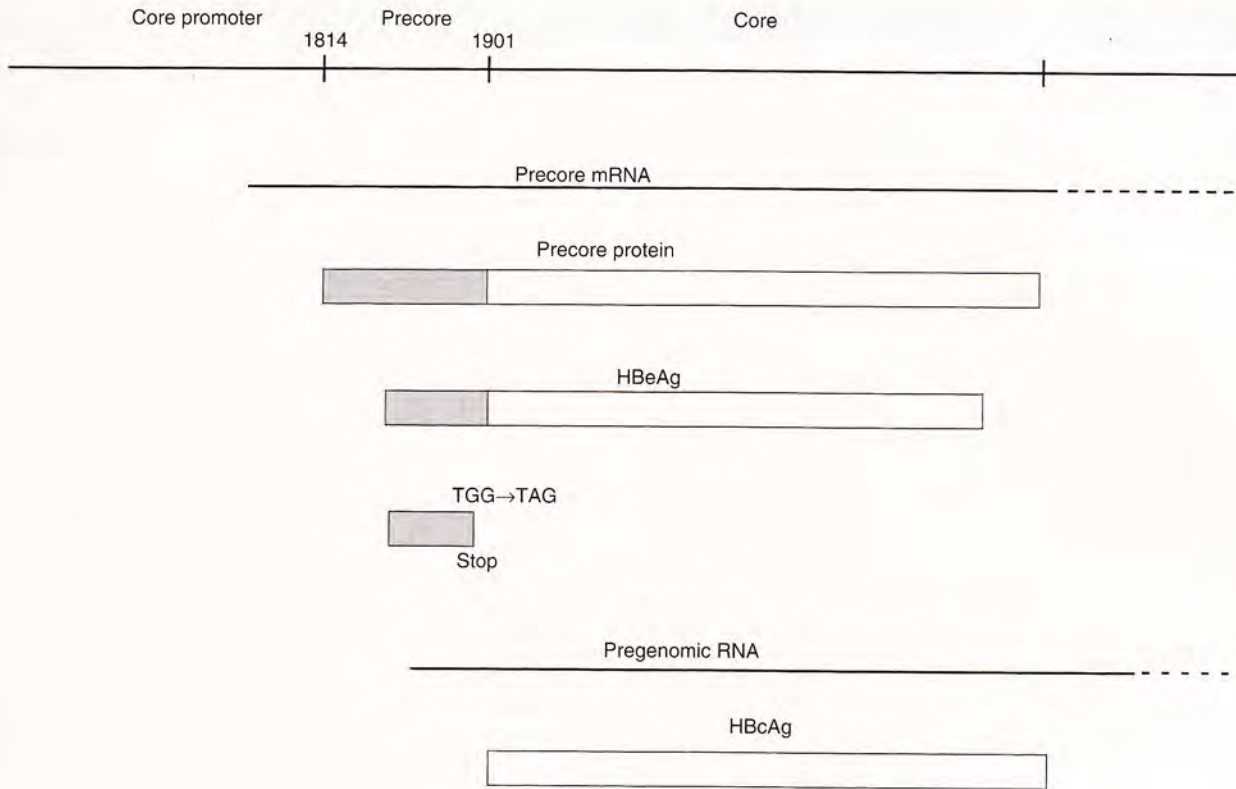
(266,346–348). This accounts for the high prevalence of HBeAg-negative chronic hepatitis and precore stop codon variant (G<sub>1896</sub>A) in Asia and the Mediterranean basin and their low prevalence in the United States and Northern Europe. The most common core promoter variant (A<sub>1762</sub>T, G<sub>1764</sub>A) is more frequently found in patients with HBV genotypes A, C, and D (266).

Several studies suggest that HBV genotypes may be related to the rate of recovery or likelihood of a fulminant course during acute infection (349–352); these studies involved small numbers of patients and the data need to be confirmed.

Studies in Asia where genotypes B and C are predominant found that genotype B is associated with a lower prevalence of HBeAg, earlier HBeAg seroconversion, higher likelihood of sustained remission after HBeAg seroconversion, and less active liver disease compared to genotype C (107,266,270,353,354). HBV genotype B is also associated with a slower rate of progression to cirrhosis and HCC (291–293,355–357). Data on the relation between other HBV genotypes and HBV replication and liver disease are scanty. Available data suggest that genotype A is associated with a higher likelihood of sustained virologic and biochemical remission after HBeAg seroconversion than genotype D (358).

HBV genotype may also impact response to IFN therapy. Several studies reported that HBeAg-positive patients with genotypes A and B have higher rates of HBeAg loss than those with genotypes C and D (359–361). There is as yet no evidence that HBV genotypes are related to IFN response among HBeAg-negative patients. Nucleoside/nucleotide analogs appear to be equally effective at virus suppression across all HBV genotypes (362). Data on the association between HBV genotypes and durability of response and rate of drug resistance are conflicting and limited by the small number of patients studied and heterogeneity in treatment regimen (363–368).





■ **Figure 29.4** Transcription and translation of precore/core open reading frame. Precore mRNA is translated into precore protein which is processed at the N and C terminal ends to hepatitis B e antigen (HBeAg). Mutation of nucleotide 1896 from G to A converts codon 28 in the precore region from tryptophan to stop codon thereby preventing production of HBeAg. Pregenomic RNA is translated into core and polymerase protein and reverse transcribed into hepatitis B virus DNA. HBcAg, hepatitis B core antigen.

## PRECORE VARIANTS

Among the naturally occurring HBV mutations, mutations in the precore region have been most extensively studied (Fig. 29.4). The precore region consists of 87 nucleotides (29 amino acids) that precede the core region. The *preC/C* gene codes for two 3.5-kb RNA transcripts. The precore mRNA is slightly longer and initiates upstream of the pregenomic RNA. It codes for a precursor protein which includes the entire precore/core gene. The nascent precore/core protein is processed at both the N and C terminal ends giving rise to a smaller secretory protein—HBeAg (367). The pregenomic RNA serves as a template for reverse transcription into the (–) strand HBV DNA. It also serves as an mRNA for translation into the core protein and polymerase protein.

The predominant mutation in the precore region is a G-A change at nucleotide 1896, creating a stop codon at codon 28 ( $G_{1896}A$ , eW28X) (138) (Table 29.9). This mutation leads to premature termination of the precore/core protein, therefore preventing the production of HBeAg. Because the precore region is not essential for HBV replication and the  $G_{1896}A$  mutation is upstream of the core region, HBV replication and

HBcAg expression are not affected. Precore variants may be selected because of their ability to evade immune clearance or to enhance HBV replication.

## Epidemiology and transmission

Initial reports of the  $G_{1896}A$  variant came from the Mediterranean countries and Japan (138,368–370). Recent studies found that this variant can be detected in diverse geographical areas (280,371,372). Although it was previously thought to be rare in North America and Western Europe, recent studies found that precore variant can be found in up to 30% patients with chronic HBV infection in the United States (282,373,374). The variability in the prevalence of the  $G_{1896}A$  variant in different countries is related to the predominant HBV genotype and the nucleotide at position 1858, located opposite 1896 in the stem-loop structure of the pregenome encapsidation sequence ( $\epsilon$ ) (371,375). The  $G_{1896}A$  variant is replication competent and infectious. Transmission has been documented in humans from infected mothers to infants, between sexual partners, and nosocomially, as well as in chimpanzee experiments (369,376–378). However, infection with precore HBV variant is less likely to progress to chronic

**TABLE 29.9. COMMON FORMS OF HEPATITIS B VIRUS (HBV) VARIANTS/MUTANTS****HBeAg NEGATIVE PHENOTYPE****Pre-core region**

G-A at nt 1896, tryptophan-stop at codon 28 (G1896A, eW28X)

G-A at nt 1899, glycine-aspartate at codon 29 (G1899A, eG29D)

**Core promoter region**

A-T at nt 1762 and G-A at nt 1764 (A1762T, G1764A, xK130M, xV131I)

**HBIG/HBV VACCINE ESCAPE****S Gene**

Glycine-arginine at codon 145 (sG145R)

**ANTIVIRAL-RESISTANCE****P gene (reverse transcriptase/polymerase region)**

Lamivudine	L180M	M204V/I	
Adefovir	A181V/T	N236T	
Entecavir	T184G	S202G/I	M250V
Telbivudine		M204I	
L180M	Leucine-methionine at codon 180		
A181V/T	Alanine-valine or threonine at codon 181		
T184G	Threonine-glycine at codon 184		
S202G/I	Serine-glycine/isoleucine at codon 202		
M204V/I	Methionine-valine or isoleucine at codon 204		
N236T	Asparagine to threonine at codon 236		
M250V	Methionine to valine at codon 250		

HBeAg, hepatitis B e antigen; HBIG, hepatitis B immune globulin; nt, nucleotide.

infection possibly due to the lack of the tolerogenic effect of HBeAg.

Many other mutations in the precore region have been reported including mutations of the start codon but they are less common and their clinical significance is less certain.

**Pathogenesis and clinical manifestations**

The G<sub>1896A</sub> variant is usually found in HBeAg-negative patients but may be present as a mixture with wild type virus in HBeAg-positive patients. It has been observed to emerge or become selected as the predominant species around the time of HBeAg seroconversion (368,370,379). The G<sub>1896A</sub> variant was initially thought to cause more severe liver disease because it was found in patients with CAH or fulminant hepatitis (138,368,369,377,378). However, some of the studies on patients with fulminant hepatitis reported that the same mutation was also found in the source patients (369,377,378). The G<sub>1896A</sub> variant has also been detected in anti-HBe-positive asymptomatic carriers (370,372,380). Therefore, the G<sub>1896A</sub> mutation alone may have no direct pathogenic role; instead host immune response and mutations in other regions of the HBV genome may be more important in determining the severity of liver disease.

**Diagnosis**

The presence of the G<sub>1896A</sub> variant should be suspected in patients who are HBsAg positive, HBeAg negative, anti-HBe positive; but continue to have elevated ALT levels, active hepatitis and HBcAg expression on liver biopsies. Confirmation that the liver disease is due to persistent HBV replication should be made by detection of HBV DNA in serum and by excluding other causes of liver disease including superimposed hepatitis C and D. Detection of the G<sub>1896A</sub> variant can be achieved by many methods including direct sequencing, restriction fragment length polymorphism (RFLP), line probe assay, ligase chain reaction, colorimetric point mutation assay, and real-time PCR assay.

**Treatment**

Anti-HBe-positive patients who have the G<sub>1896A</sub> variant have been reported to have lower rates of sustained response to IFN as well as nucleoside/nucleotide treatment compared to HBeAg-positive ones (381). The role of the G<sub>1896A</sub> variant in response to IFN therapy in patients with HBeAg-positive chronic hepatitis is less clear. One study reported that HBeAg-positive patients who had G<sub>1896A</sub> variant either as the predominant species or as a mixture with wild type HBV had higher rates of response (379). It is possible that these patients

were on the way to spontaneous HBeAg seroconversion and IFN therapy merely hastened the process. There is no evidence that antiviral treatment induces a higher rate or unique pattern of mutations in the precore region (379).

### CORE PROMOTER MUTATIONS

Mutations in the core promoter region can also prevent HBeAg production without affecting HBV replication or HBeAg expression by selectively downregulating the transcription of the precore mRNA but without affecting the pregenomic RNA (382,383). The most common core promoter variant had dual mutations involving A to T change at nucleotide 1762 and G to A change at nucleotide 1764 (A<sub>1762</sub>T, G<sub>1764</sub>A) (Table 29.9). These two changes result in amino acid substitutions in codons 130 and 131 of the overlapping X gene. Unlike precore variants, core promoter variants can be detected in similar proportions of HBeAg-negative and HBeAg-positive patients (281,282,384,385). One study demonstrated that emergence of the core promoter changes was more common among HBeAg-positive patients who subsequently seroconvert compared to those who remained HBeAg positive (386), indicating that the selection of the core promoter variant may play a role in HBeAg clearance. Core promoter variants are more commonly found in patients with CAH, fulminant hepatitis or HCC (308,387–391). Core promoter mutations have been shown in several studies to be associated with HCC, whether this is related to more active liver disease, frequent association with genotype C, or mutations in the overlapping X gene is unclear.

### CORE GENE MUTATIONS

The HBV core protein is essential for virus replication. Mutations in the HBV core gene have been detected in patients with chronic HBV infection (392–395). Mutations in the core gene are more often reported in association with older age, HBeAg negativity, active liver disease and presence of the precore variant (392). Longitudinal studies found that most of the mutations occur around the time of HBeAg seroconversion or exacerbations of chronic hepatitis (396). Naturally occurring mutations involving critical residues in the HLA-A2 restricted CTL epitope (amino acids 18 to 27) leading to T-cell receptor antagonism have been reported (397). However, CTL escape mutant is not the cause of persistent infection in most of the patients with chronic HBV infection (398).

### PRE-S/S REGIONS

The pre-S/S region codes for two RNA transcripts that are separately regulated by the pre-S1 promoter and the

pre-S2/S promoter. The larger transcript encompassing the pre-S1, pre-S2, and S regions is translated into the large S protein. The smaller transcript encompassing the pre-S2 and S regions is translated into the middle S and small S proteins. The large S protein is essential for virion formation. The middle S protein is dispensable. The small S protein, HBsAg, is the major envelope protein. The “a” determinant of HBsAg is the predominant B cell epitope, and is common to all HBV serotypes. It is a conformational epitope.

### S GENE MUTATIONS

#### Vaccines

Mutations in the S gene have been described in infants born to carrier mothers despite adequate anti-HBs response after vaccination (399–401). The most common mutation involves a glycine to arginine substitution at codon 145 (G145R) in the “a” determinant (Table 29.9). This mutation has been shown to exhibit decreased binding to monoclonal anti-“a” antibodies thereby allowing the virus to escape neutralization. The G145R mutant has now been reported in many countries. Chimpanzee experiments demonstrated that this mutant is infectious and pathogenic (402). These findings raise concerns about the possibility of widespread dissemination of vaccine escape mutants. A recent study from Taiwan found that the prevalence of HBV S mutants among HBsAg-positive children increased after the introduction of universal vaccination, from 7.8% in 1984 to 23% in 1999 (403–405). However, the carrier rate among Taiwanese children decreased from 9.8% to 0.7% (31) during the same period. Another study among 784 preschool children in four Pacific Island countries, who received HBV vaccine failed to detect any “a” determinant mutation (406). These data suggest that the emergence of vaccine escape mutants occurs rarely and has not diminished the efficacy of HBV vaccines. Apart from the G145 R mutant, other mutations in the ‘a’ determinant have also been reported in lower frequencies (407). Many of these mutants have also been shown to reduce binding to monoclonal anti-“a” antibodies. This raises concerns that some of the S mutants may yield false negative results in serology assays. However, practically all the S mutants that have been identified to date can be detected in HBsAg assays that utilize polyclonal anti-HBs for capture and/or detection.

#### Liver transplant recipients

Mutations in the HBV S gene have also been reported in liver transplant recipients who developed HBV reinfection despite prophylaxis with monoclonal or polyclonal anti-HBs (HBIG) (408–410). Most of the mutations are

located in the “a” determinant, the most common being the G145R mutation. One study found a direct correlation between the incidence of the G145R mutant and the duration of HBIG administration, and reversal of the mutation after cessation of HBIG (410). Prophylaxis with combination of HBIG and lamivudine decreases the risk of immune escape and rate of reinfection (411–414).

### PRE-S MUTATIONS

Mutations in the pre-S1 and pre-S2 regions have also been described in patients with chronic HBV infection (415–417). The prevalence and the clinical significance of most of these mutations are uncertain. Several investigators reported a high incidence of deletions or mutations in pre-S1 that may cause dysregulation of small S protein synthesis and secretion (418). Retention of HBsAg has been demonstrated to induce liver injury and HCC in transgenic mice (174).

### P GENE MUTATIONS

The HBV polymerase gene consists of four distinct regions: A primer involved in priming of reverse transcription, a spacer with no known function, a reverse transcriptase/DNA polymerase which is responsible for reverse transcription of the pregenomic RNA into the first (–) strand HBV DNA and for synthesis of the second (+) strand HBV DNA, and an RNase H which removes the RNA template (419). The reverse transcriptase/DNA polymerase region has five conserved domains: A, B, C, D, E. Domains A, C and D are involved in nucleoside triphosphate binding and catalysis, whereas domains B and E participate in the positioning of the RNA template and the primer relative to the catalytic site (420,421). The putative catalytic domain is believed to reside in the tyrosine–methionine–methionine–aspartate (YMDD) locus in domain C. This locus is conserved in all viral reverse transcriptases as well as in all isolates of hepadnaviruses (419). Naturally occurring HBV polymerase gene mutations are rarely reported. The most common *P* gene mutations have been found in association with the use of nucleoside/nucleotide analogs for the treatment of chronic HBV infection.

### X GENE MUTATIONS

The HBV *X* gene regulates HBV replication through activating and regulating viral and cellular genes. Several studies found that mutations affecting codons 130 and 131 of the *X* gene are more common in patients with HCC (308,390,391) but these *X* gene mutations correspond to the dual core promoter mutations A<sub>1762</sub>T, G<sub>1764</sub>A, which have also been found in many

patients with nonmalignant HBV-related chronic liver disease. Therefore, the role of *X* gene mutations in the development of HCC remains to be established.

## Treatment

The main goal of treatment of chronic hepatitis B is to prevent cirrhosis, hepatic failure and HCC. This goal is best achieved by eradicating HBV before there is irreversible liver damage. However, it is not possible to eradicate HBV because of the presence of extrahepatic reservoirs of HBV, the integration of HBV DNA into the host genome, and the presence of an intracellular conversion pathway that replenishes the pool of transcriptional templates (covalently closed circular HBV DNA [cccDNA]) in the hepatocyte nucleus without the need for reinfection (253). Currently, there are five approved treatments for hepatitis B: Standard and pegylated IFN- $\alpha$ , and three nucleoside/nucleotide analogs—lamivudine, adefovir, and entecavir. Many new antiviral and immunomodulatory therapies are being evaluated; some have shown promise and may play a key role in the treatment of chronic HBV infection (Table 29.10).

### DEFINITION OF RESPONSE AND ENDPOINTS OF TREATMENT

At the 2000 National Institutes of Health workshop on Management of Hepatitis B, it was proposed that definition and criteria of response to antiviral therapy of chronic hepatitis B be standardized. The proposal categorized responses as biochemical (BR), virological (VR), or histologic (HR), and as on-therapy or sustained off-therapy (Table 29.11) (141). The increased availability of sensitive PCR assays for quantification of HBV DNA has called for more stringent definition of virological response, suppression of serum HBV DNA to

**TABLE 29.10. TREATMENT OF CHRONIC HEPATITIS B**

#### APPROVED TREATMENTS IN THE UNITED STATES

Standard Interferon- $\alpha$  2b (Intron A)  
Pegylated Interferon- $\alpha$  2a (Pegasys)  
Lamivudine (Epivir, 3TC)  
Adefovir dipivoxil (Hepsera)  
Entecavir (Baraclude)

#### TREATMENTS APPROVED FOR HUMAN

##### IMMUNODEFICIENCY VIRUS WITH EFFICACY AGAINST HEPATITIS B VIRUS

Emtricitabine (Emtriva, FTC)  
Tenofovir (Viread)  
Emtricitabine + Tenofovir (Truvada)

U

V

W

**TABLE 29.11. DEFINITION OF RESPONSE TO ANTIVIRAL THERAPY OF CHRONIC HEPATITIS B**

CATEGORY OF RESPONSE	
Biochemical (BR)	Decrease in serum ALT to within the normal range
Virological (VR)	Decrease in serum HBV DNA to $<10^5$ IU/mL and loss of HBeAg in patients who were initially HBeAg positive; undetectable serum HBV DNA ( $<10^2$ IU/mL) in patients who were initially HBeAg negative
Histologic (HR)	Decrease in histologic activity index by at least two points compared to pretreatment liver biopsy
Complete (CR)	Fulfill criteria of biochemical and virological response and loss of HBsAg

**TIME OF ASSESSMENT**

On-therapy	During therapy
Maintained	Persist throughout the course of treatment
End-of-treatment	At the end of a defined course of therapy
Off-therapy	After discontinuation of therapy
Sustained (SR-6)	6 mo after discontinuation of therapy
Sustained (SR-12)	12 mo after discontinuation of therapy

HBV, hepatitis B virus; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

Adapted from Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000—summary of a workshop. *Gastroenterology* 2001;120(7):1828–1853.

undetectable level by PCR ( $<10^2$  IU/mL), particularly for patients with HBeAg-negative chronic hepatitis.

Although IFNs are administered for predefined durations, nucleoside/nucleotide analogs are usually administered until specific endpoint is achieved. The difference in approach is related to the observation that HBeAg seroconversion frequently occurs a few months after cessation of IFN treatment, presumably because of the lag between immune priming and decrease in expression of viral protein; viral rebound is inevitable if nucleoside/nucleotide analogs are withdrawn prior to achieving therapeutic endpoint. For HBeAg-positive patients, virus suppression can be sustained in 50% to 90% patients if treatment is stopped after HBeAg seroconversion is achieved. For HBeAg-negative patients, relapse is frequent even when HBV DNA has been suppressed to undetectable levels by PCR assays for more than a year; therefore, the endpoint for stopping treatment is unclear.

**INTERFERONS**

IFNs have antiviral, antiproliferative and immunomodulatory effects. IFN- $\alpha$  and - $\beta$  bind to the same receptor and have predominantly antiviral effects, whereas IFN- $\gamma$  binds to a separate receptor and has more marked

immunoregulatory but less potent antiviral effects. The antiviral effects of IFN depend on its binding to specific receptors, which then triggers a series of intracellular events including activation of 2'5'-oligoadenylate synthetase (2'5'-AS). IFNs also have immunomodulatory functions that may be important in eradicating virus infections.

**STANDARD INTERFERON- $\alpha$** **Efficacy**

1. *HBeAg-positive chronic hepatitis.* Clinical studies confirmed that IFN- $\alpha$  suppresses HBV replication. A positive response as defined by loss of viral replication markers (HBeAg and serum HBV DNA by unamplified assays) within 12 months of initiation of treatment can be achieved in 30% to 40% of HBeAg-positive patients who have elevated ALT (Table 29.12) (155,422–425). Loss of HBsAg is less common and occurs in 5% to 10% of patients only. It should be noted that spontaneous loss of HBeAg can occur in 5% to 15% of untreated controls. Therefore, a treatment-related benefit is only seen in approximately 20% to 25% of patients. Despite the wide range in response rates in reported studies, several meta-analyses confirmed a beneficial effect of IFN therapy (426,427).
  - a) *HBeAg-positive patients with normal ALT.* This pattern is usually seen in children or young adults who are in the immune tolerance phase of HBV infection. HBeAg seroconversion occurs in less than 10% of these patients (428–431).
  - b) *Oriental patients.* Asian patients with elevated ALT levels have comparable rates of HBeAg seroconversion as white patients (429). However, a higher proportion of Asian patients have normal ALT levels, so overall response is poor if treatment is extended to include those with normal ALT levels.
  - c) *Children.* Ideally, treatment should be instituted as early as possible before there is irreversible liver damage. Among children with elevated ALT, HBeAg clearance has been reported in 30% of those who received IFN- $\alpha$  compared to 10% of controls (432–435). However, most children, particularly those with perinatally acquired HBV infection have normal ALT and less than 10% of these children who received IFN- $\alpha$  cleared HBeAg (430,431).
2. *HBeAg-negative chronic hepatitis.* Results of four randomized controlled trials involving a total of 86 IFN- $\alpha$  treated patients and 84 controls showed that the end-of-treatment response ranged from 38% to 90% in treated patients compared to 0% to 37% of controls (436–439). A major problem with

IFN- $\alpha$  treatment of HBeAg-negative chronic hepatitis is the high rate of relapse. Approximately 50% of the responders relapse when therapy is discontinued and relapses can occur up to 5 years post-therapy. A study from Italy reported a higher rate (30%) of sustained response after 24 months of IFN- $\alpha$  therapy compared to 15% to 20% rates reported in other studies that administered IFN- $\alpha$  for 12 months (438) suggesting that longer duration of treatment may increase the rate of sustained response.

3. *HBV DNA positive clinical cirrhosis.* Patients with histologic cirrhosis but no evidence of hepatic decompensation appeared to tolerate IFN- $\alpha$  treatment and responded as well as patients with pre-cirrhotic chronic hepatitis B (155,429). However, IFN- $\alpha$  even when administered in very low doses (3 MU thrice weekly) is associated with a high risk of serious infections and severe exacerbations leading to hepatic failure in patients with clinically evident cirrhosis (440,441).
4. *Nonresponders to IFN- $\alpha$  treatment.* Most studies found that IFN- $\alpha$  retreatment of HBeAg-positive patients who previously failed to respond to IFN- $\alpha$  was associated with a very low rate of response. One trial of 57 HBeAg-positive patients reported an HBeAg clearance rate of 33% among patients retreated with IFN- $\alpha$  versus 10% in untreated controls (442). However, this trial included patients who were previously treated with suboptimal doses of IFN- $\alpha$  and may have overestimated the benefits of IFN- $\alpha$  retreatment.

Limited data suggest that 20% to 30% HBeAg-negative patients who relapsed or had no response during previous IFN- $\alpha$  treatment had a sustained response after a second course of IFN- $\alpha$  (443).

### Role of prednisone priming

Steroid withdrawal has been observed to be frequently accompanied by a flare in serum ALT levels and HBeAg seroconversion. A meta-analysis of seven studies that examined prednisone priming followed by IFN- $\alpha$  treatment (when immune response recovers) found that prednisone priming had very little additional beneficial effect compared to IFN- $\alpha$  alone (444). A subsequent study of 200 European patients reported that patients who received prednisone priming had a significantly higher rate of HBeAg seroconversion compared to those who received IFN- $\alpha$  alone (445). The overall data suggest that a small subset of patients may benefit from prednisone priming but there is a risk of fatal exacerbation in patients with underlying cirrhosis. With the availability of new treatment options, prednisone priming is not recommended for patients with chronic hepatitis B.

### Long-term outcome of therapy with interferon- $\alpha$

#### *Hepatitis B e antigen-positive patients*

Most HBeAg-positive patients who responded to IFN- $\alpha$  therapy are able to maintain their response unless they become immunocompromised. Delayed reactivation has been observed in 10% to 20% of responders only, most of which occurred within 1 year of cessation of treatment (154,446). Nevertheless, complete disappearance of markers of HBV replication as determined by PCR assay for serum HBV DNA is seldom achieved in patients who cleared HBeAg only. In addition, several studies reported that the 5-year cumulative rates of HBeAg clearance were similar in treated patients and controls (447–449) suggesting that the main role of IFN- $\alpha$  may be to reduce the duration of active liver disease by hastening viral clearance.

Among the sustained responders, an increasing proportion cleared HBsAg during the course of follow-up. However, the percent of sustained responders who cleared HBsAg within 5 years of HBeAg clearance varied from 65% in one US study (446) to 19% to 24% in European studies (447,450–452) to 0% to 9% in two studies from Asia (154,453) despite similar durations of follow-up. A sustained antiviral response, especially in those who clear both HBeAg and HBsAg, is almost invariably accompanied by normalization of ALT levels and decrease in necroinflammation but residual liver damage may be present. Data to substantiate the hypothesis that a sustained antiviral response can lead to decreased risks of cirrhosis and HCC and improved survival are limited because chronic hepatitis B is an insidious disease and complications may not be evident until decades later. In addition, patients initially randomized to the control group frequently receive treatment after completion of the trial. There has been only one report comparing the outcome of IFN- $\alpha$ -treated patients and controls. An 8-year follow-up of 101 male patients (67 IFN- $\alpha$ -treated and 37 untreated controls) who participated in a controlled trial of IFN- $\alpha$  therapy in Taiwan found that treated patients had a lower incidence of HCC (1.5% vs. 12%,  $P = 0.04$ ) and a higher survival rate (98% vs. 57%,  $P = 0.02$ ) but there was no difference in the rate of new onset of cirrhosis (453). However, clinical benefits were not observed in a follow-up report from Hong Kong of 208 IFN- $\alpha$  treated and 203 untreated controls (449). IFN- $\alpha$  has not been shown to decrease the incidence of HCC in European or North American patients probably because of the low rate of HCC in untreated patients but studies comparing the outcome of responders vs nonresponders found that patients who cleared HBeAg had better overall survival and survival free of hepatic decompensation; a clinical benefit was most evident among patients with cirrhosis (427,447,451,454,455).

### Hepatitis B e antigen–negative patients

Contrary to HBeAg-positive patients, relapse after cessation of IFN- $\alpha$  treatment is frequent, with sustained response rates of only 15% to 30% (443,456). Among the long-term responders, approximately 20% cleared HBsAg after 5 years of follow-up (457). IFN- $\alpha$  therapy did not have any overall effect on survival, complication-free survival or HCC but patients with sustained response had significantly lower rates of hepatic decompensation (456,457).

### Dose regimen

IFN- $\alpha$  is administered as subcutaneous injections. The recommended dose for adults is 5 MU daily or 10 MU thrice weekly and for children 6 MU/m<sup>2</sup> thrice weekly with a maximum of 10 MU.

### Hepatitis B e antigen–positive patients

The recommended duration of treatment for patients with HBeAg-positive chronic hepatitis B is 16 to 24 weeks. There are very little data to support the use of a longer duration of IFN- $\alpha$  treatment in HBeAg-positive patients. One study reported that among patients who have not cleared HBeAg after 16 weeks of IFN- $\alpha$ , those randomized to continue treatment until week 32 had significantly higher rates of HBeAg clearance compared to those who stopped treatment (458). However, other studies found that response rates after 24 or 12 weeks of IFN- $\alpha$  therapy were similar.

### Hepatitis B e antigen–negative patients

Current data suggest that patients with HBeAg-negative chronic hepatitis B should be treated with IFN- $\alpha$  for at least 12 months; one study suggested that higher rates of sustained response can be achieved with 24

months of treatment but that study did not include a comparison group with 12 months treatment (438).

## PEGYLATED INTERFERON- $\alpha$

The attachment of polyethylene glycol to a protein (pegylation) reduces its rate of absorption following subcutaneous injection, reduces renal clearance, and decreases immunogenicity of the protein, with resultant increase in the half-life of the pegylated protein. PegIFN- $\alpha$  has the advantage of more convenient administration and more sustained virus suppression. Clinical trials suggest that efficacy of pegIFN- $\alpha$  is similar or slightly better than standard IFN- $\alpha$ .

### Efficacy

1. *HBeAg-positive chronic hepatitis.* In one phase II trial, 194 patients were randomized to receive 90, 180 or 270  $\mu$ g pegIFN- $\alpha$ 2a weekly or 4.5 MU standard IFN- $\alpha$ 2a thrice weekly for 24 weeks (Table 29.12). A higher percent of patients who received pegIFN- $\alpha$  had HBeAg seroconversion, 32% versus 25% of those who received standard IFN- $\alpha$  (459). Although this study used a suboptimal dose of standard IFN- $\alpha$ , the convenience of once weekly dosing has led to the replacement of standard IFN with pegIFN. In a subsequent phase III trial, 814 patients were randomized to receive pegIFN- $\alpha$ 2a 180  $\mu$ g weekly, pegIFN- $\alpha$ 2a 180  $\mu$ g weekly + lamivudine 100 mg daily, or lamivudine 100 mg daily for 48 weeks. At the end of treatment, virus suppression was most marked in the group that received combination therapy, mean HBV DNA reduction in the three groups was 4.5, 7.2, and 5.8 log<sub>10</sub> copies/mL, respectively (460). HBeAg seroconversion was similar in the three groups at the end of treatment: 27%, 24%, and 20%, respectively, but significantly

**TABLE 29.12. COMPARISON OF PERCENTAGES OF PATIENTS WITH ANTIVIRAL RESPONSES TO APPROVED TREATMENTS OF HEPATITIS B**

Response	Standard IFN	Pegylated IFN	Lamivudine	Adefovir	Entecavir	No treatment
<b>HBeAg+ patients</b>	<b>12–24 wk</b>	<b>48 wk</b>	<b>52 wk</b>	<b>48 wk</b>	<b>48 wk</b>	<b>48–52 wk</b>
Undetectable HBV DNA	NA	10–25/7–14	40–44	21	67	0–16
HBeAg seroconversion	~15/~20	22–27/29–32	16–18	12	21	4–10
Loss of HBsAg	1–8	3	<1	0	NA	0–2
Normalization of ALT	~20	34–39/32–41	41–72	48	68	7–24
Histologic improvement	NA	38	49–56	53	72	~25
Durability of response	80–90	~80	50–80	~90 <sup>a</sup>	~80	NA
<b>HBeAg– patients</b>	<b>6–12 mo</b>	<b>48 wk</b>	<b>52 wk</b>	<b>48 wk</b>	<b>48 wk</b>	<b>48–52 wk</b>
Undetectable HBV DNA	~60	63/19	50–70	51	90	<10
Normalization of ALT	60–70	38/59	60–70	72	78	~30
Histologic improvement	NA	60	64	70	48	30–40
Durability of response	~20	15	<10	<10	NA	NA

<sup>a</sup>Median duration of treatment = 80 wk.

IFN, interferon; HBV, hepatitis B virus; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

higher in the two groups that received pegIFN- $\alpha$  when response was assessed 24 weeks after treatment was stopped: 32%, 27%, and 19%, respectively. These data indicate that pegIFN- $\alpha$ 2a monotherapy was superior to lamivudine monotherapy in inducing HBeAg seroconversion, and comparable to the combination therapy of pegIFN- $\alpha$ 2a and lamivudine. Similar results were reported in two trials, in which pegIFN- $\alpha$ 2b was administered in tapering doses (100  $\rightarrow$  50  $\mu$ g) for 52 weeks or 1.5  $\mu$ g/kg for 32 weeks. Twenty-four weeks after treatment was stopped, one study reported identical rates (29%) of HBeAg seroconversion in patients who received pegIFN- $\alpha$ 2b monotherapy with and without lamivudine (359), whereas the other study reported a significantly higher rate of HBeAg seroconversion in those who received a combination of pegIFN- $\alpha$ 2b and lamivudine versus those who received lamivudine only, 36% versus 14% (461). Follow-up of patients in the latter study found that a significant difference in HBeAg seroconversion was maintained up to 76 weeks after treatment was stopped (462).

2. *HBeAg-negative chronic hepatitis*. In the only published report of peg IFN- $\alpha$  in HBeAg-negative patients, 552 patients were randomized to receive 48 weeks of pegIFN- $\alpha$ 2a 180  $\mu$ g weekly, combination of pegIFN- $\alpha$ 2a 180  $\mu$ g weekly + lamivudine 100 mg daily, or lamivudine 100 mg daily. As in HBeAg-positive patients, virus suppression was most marked in the group that received combination therapy, mean HBV DNA reduction at week 48 in the three groups was 4.1, 5.0, and 4.2 log<sub>10</sub> copies/mL, respectively (463). However, sustained response (HBV DNA undetectable by PCR and normalization of ALT at week 72) was comparable in the groups that received pegIFN- $\alpha$ 2a alone or in combination with lamivudine, and superior to the group that received lamivudine monotherapy: 15%, 16%, and 6%, respectively.

### Dose regimen

Currently, only pegIFN- $\alpha$ 2a is approved for the treatment of chronic hepatitis B. The recommended dose is 180  $\mu$ g weekly for 48 weeks. However, given the similarity in response rates between 90 and 180  $\mu$ g doses in the phase II trial, and the comparable response rates between 24 and 48 week treatment in the phase II and phase III trials (459,460), it is possible that lower doses and/or shorter duration of treatment may suffice for HBeAg-positive patients. Whether longer duration of treatment (>48 week) will result in higher rates of sustained response in HBeAg-negative patients remains to be determined.

Although the two formulations of pegIFN- $\alpha$  likely have similar efficacy, the optimal dose and duration of pegIFN- $\alpha$ 2b for hepatitis B is unclear.

### Adverse effects of interferon- $\alpha$ therapy

IFN- $\alpha$  therapy is associated with a broad spectrum of side effects (464). The most common side effect is an initial influenza-like illness: Fever, chills, headache, malaise and myalgia. Other common side effects include fatigue, anorexia, weight loss and mild increase in hair loss. IFN- $\alpha$  has myelosuppressive effects but significant neutropenia (<1,000/mm<sup>3</sup>) or thrombocytopenia (<60,000/mm<sup>3</sup>) requiring dose reduction or premature termination are uncommon except in patients who have decreased cell counts prior to treatment. The most troublesome side effect of IFN- $\alpha$  is emotional lability. Anxiety, irritability, depression and even suicidal tendency. These symptoms can occur in the absence of a prior history of emotional problems. IFN- $\alpha$  has been reported to induce the development of a variety of autoantibodies. In most instances, this is not accompanied by clinical illness. However, both hyper- and hypothyroidism that require treatment have been reported. In addition, there have been reports of worsening liver disease as a result of IFN- $\alpha$  induced exacerbation of an underlying autoimmune hepatitis. Rarely have retinal changes and even impaired vision been reported. Side effects of pegIFN- $\alpha$  are similar to that of standard IFN- $\alpha$ .

### Predictors of response to standard and pegylated interferon- $\alpha$

Predictors of response to standard and pegIFN- $\alpha$  are similar. The strongest predictor of response in HBeAg-positive patients is pretreatment ALT level (429,465). Other factors that have been identified to be associated with a higher rate of IFN-related HBeAg seroconversion include high histologic activity index, low HBV DNA level, and more recently HBV genotypes A and B (359–361). There is no consistent predictor of sustained response among HBeAg-negative patients. Some studies found that rapid normalization of ALT and high titer IgM anti-HBc are associated with a higher rate of sustained response but they have not been validated by other studies.

### INTERFERON- $\beta/\gamma$

Early studies using IFN- $\beta$  from fibroblast cultures were disappointing. More encouraging results were obtained using recombinant IFN- $\beta$ , but the data are limited. IFN- $\gamma$  can inhibit HBV replication, but the antiviral efficacy is less than that of IFN- $\alpha$ . In vitro studies suggest that IFN- $\gamma$  may have anti-fibrotic effects but its *in vivo* efficacy remains to be proven. Combination therapy of IFN- $\gamma$  with IFN- $\alpha/\beta$  has no additional antiviral effects.



## LAMIVUDINE (EPIVIR-HEPATITIS B VIRUS, TRIPHOSPHATE)

Lamivudine is the (–) enantiomer of 2',3'-dideoxy-3'-thiacytidine. It is phosphorylated to the triphosphate (3TC-TP) which competes with dCTP for incorporation into growing DNA chains causing chain termination. This may occur during reverse transcription of the first strand as well as during synthesis of the second strand HBV DNA (466).

### Efficacy

Lamivudine monotherapy is effective in suppressing HBV replication and in ameliorating liver disease (Table 29.12). One-year treatment with lamivudine results in similar rate of HBeAg seroconversion as 16 week of standard IFN- $\alpha$ , but is inferior to a 1-year course of pegIFN- $\alpha$ . Longer duration of treatment is associated with higher rates of HBeAg seroconversion but also increasing rates of drug-resistant mutations.

1. *HBeAg positive chronic hepatitis B.* Three randomized clinical trials involving a total of 731 patients who received lamivudine for 1 year reported that HBeAg seroconversion occurred in 16% to 18% of treated patients compared to 4% to 6% of untreated controls (467–469). Histologic improvement defined as reduction in necroinflammatory score 2 points or greater was observed in 49% to 56% treated patients and in 23% to 25% controls. HBeAg seroconversion rates increased with the duration of treatment to approximately 50% at 5 years (470–473). Whether the incremental HBeAg seroconversion can be attributed to the additional years of lamivudine treatment is unclear because an untreated control group was not available for comparison.
  - a) *HBeAg-positive patients with normal ALT.* As with IFN- $\alpha$  therapy, HBeAg seroconversion rate is less than 10% in patients with pretreatment ALT less than two times normal after 1 year and 19% after 3 years of treatment (156,465,471).
  - b) *Oriental patients.* Oriental patients respond similarly to lamivudine as whites (465,467–469).
  - c) *Children.* Experience with lamivudine in children is limited. One controlled trial involved 286 children, aged 2 to 17 years, with ALT more than 1.3 times normal randomized in a 2:1 ratio to receive lamivudine (3 mg/kg per day up to 100 mg/day) or placebo for 52 weeks. A significantly higher proportion of treated children developed HBeAg seroconversion compared to placebo controls, 22% versus 13% (474). As with adults, HBeAg seroconversion rate was higher among children with pretreatment ALT greater than three times normal: 34% versus 16% than those with ALT
2. *HBeAg-negative chronic hepatitis B.* Lamivudine has been shown to benefit patients with HBeAg-negative chronic hepatitis B (475–479). In the only placebo-controlled study, virological and biochemical response (HBV DNA undetectable by bDNA assay and normalization of normal ALT) was achieved in 34 of 54 (63%) patients who received 24 weeks of lamivudine therapy versus 3 of 53 (6%) patients on placebo ( $P < 0.001$ ) (475). Several studies have shown that HBV DNA is suppressed to undetectable levels by PCR assay in 60% to 70% patients after 1 year of treatment. However, the vast majority (approximately 90%) of patients relapsed when treatment was stopped (476). Extending the duration of treatment results in progressively lower rates of maintained response due to selection of drug-resistant mutants. In one study of 78 patients, virologic remission (HBV DNA undetectable by PCR assay) decreased from 77% at 1 year to 52% at 2 years and 42% at 3 years; the corresponding rates of biochemical remission were 90%, 63%, and 53%, respectively (480).
3. *Nonresponders to IFN- $\alpha$  treatment.* In a multicenter trial on IFN- $\alpha$  nonresponders, 238 HBeAg-positive patients were randomized to receive lamivudine monotherapy for 52 weeks, combination of 24 weeks lamivudine and 16 weeks of standard IFN- $\alpha$  or no treatment. Patients who received lamivudine monotherapy had the highest HBeAg seroconversion rate, 18% compared to 12% and 13%, respectively in the other groups (481). These data suggest that patients who failed IFN- $\alpha$  treatment have similar response to lamivudine as treatment-naïve patients and retreatment with combination of standard IFN- $\alpha$  and lamivudine did not confer any added benefit compared to retreatment with lamivudine monotherapy.
4. *Advanced liver disease.* Lamivudine has been shown to delay clinical progression in patients with advanced fibrosis or cirrhosis by significantly reducing the incidence of hepatic decompensation and HCC. In a double blind, randomized placebo controlled trial, 651 Asian patients who were HBeAg positive or had detectable HBV DNA by branched DNA assay ( $>700,000$  genome equivalents/mL), and bridging fibrosis or cirrhosis on liver biopsy were randomized to receive lamivudine or placebo in a 2:1 ratio. After a median duration of 32 months, a statistically significant difference was observed between the two groups for overall disease progression (increase in Child-Pugh score, hepatic decompensation or HCC): 7.8% versus 17.7%; as

less than or equal to two times normal: 12% versus 8%. Lamivudine was well tolerated but the benefit must be carefully balanced against a high (19%) rate of drug resistant mutations.

well as HCC development: 3.9% versus 7.4% (332). The benefit was observed mainly among the patients who did not have breakthrough infection. These data indicate that antiviral therapy can improve clinical outcome in patients who have maintained virus suppression. Whether a similar benefit can be observed in patients who have lower serum HBV levels is unclear. It is also uncertain if clinical benefit can be maintained if treatment is stopped after HBV has been suppressed for several years.

5. *HBV DNA-positive clinical cirrhosis.* Lamivudine does not have myelosuppressive effect and rarely induces significant ALT flares during treatment. Therefore, it is a safer treatment than IFN- $\alpha$  in patients with decompensated cirrhosis. In one study of 35 patients (10 with Child's C and 25 with Child's B), improvement in liver disease defined as a decrease in Child-Pugh score of 2 or more was observed in 22 of 23 patients who received a minimum of 6 months treatment (482). However, seven patients had progressive liver disease necessitating liver transplant and an additional five died during the first 6 months. Among the initial responders, two had since died and three had developed breakthrough infection. These data indicate that clinical improvement is slow. The delay in clinical benefit was confirmed in a retrospective analysis of 154 patients who received lamivudine for HBsAg-positive decompensated cirrhosis (483). Of the 32 deaths, 25 (78%) occurred during the first 6 months. Among the patients who survived more than 6 months, the estimated 3-year actuarial survival was 88%. Multivariate analyses showed that high pretreatment bilirubin, creatinine, and HBV DNA levels were significantly associated with 6-month mortality.

Data from these and other studies demonstrate that lamivudine is safe and can stabilize or improve liver function in patients with decompensated cirrhosis thereby obviating or delaying the need for liver transplant (482–485). However, these studies showed that HCC can still occur even among patients with clinical improvement; therefore, continued surveillance is warranted.

## Predictors of response

### *Hepatitis B e antigen-positive patients*

Pretreatment serum ALT is the strongest predictor of response. Multivariate analysis of the data from the multicenter Asian study found that lamivudine treatment and pretreatment serum ALT but not pretreatment serum HBV DNA level or histologic activity index correlated with HBeAg seroconversion (156). Pretreatment serum ALT remained the most important

predictor of response when data from four studies with a total of 406 patients who received lamivudine for 1 year were pooled for analysis. HBeAg seroconversion occurred in 2%, 9%, 21%, and 47% patients with pretreatment ALT levels within normal, one to two times normal, two to five times normal and more than five times normal; while the corresponding figures for 196 patients in the placebo group were 0%, 5%, 11%, and 14%, respectively (465).

### *Hepatitis B e antigen-negative patients*

There are no data on predictors of response to lamivudine treatment of HBeAg-negative patients.

## Durability of response

Durability of HBeAg seroconversion has been reported to vary from 50% to 80%. In a follow-up report of patients in the United States and Europe, who completed 1 year of lamivudine treatment, 30 of 39 (77%) patients with HBeAg seroconversion had a durable response after a median follow-up of 37 months (range 5 to 46 months) (486). In addition, 8 (20%) patients developed HBsAg seroconversion. The estimated durability of lamivudine-induced HBeAg seroconversion was lower, 64% at 36 months, based on intention to treat analysis. Studies from Asia reported lower rates of durability—50% to 60% (365,487–490), in part related to a shorter duration of treatment, mean 8 to 9 months. Several factors have been identified to be associated with increased durability of HBeAg seroconversion including longer duration of consolidation treatment (continued treatment after HBeAg seroconversion), younger age, lower HBV DNA level at the time treatment was stopped, and genotype B versus C. The most consistent factor appears to be duration of consolidation treatment (365,488–490). Although there are no good direct comparison data, it appears that durability of lamivudine-induced HBeAg seroconversion is less than that for IFN (491).

Among HBeAg-negative patients, durability of virus suppression after 1-year of lamivudine treatment is less than 10%. One study reported that durability of virologic response (undetectable HBV DNA by PCR assay) can be improved to 50% in patients who have completed 2 years treatment and had undetectable HBV DNA by PCR assay on at least three consecutive occasions (3 months apart) (492). Confirmation of these data may help in identifying a subset of patients who do not need to be on life-long treatment.

## Lamivudine resistance

Selection of lamivudine resistant mutants is the main concern with lamivudine treatment. The most common mutation affects the YMDD motif of the HBV DNA

polymerase (M204V/I). This mutation is frequently accompanied by L180M (493,494), other mutations that have been commonly observed include V173L and changes at L80. Lamivudine resistance is usually manifested as breakthrough infection defined as greater than 1 log<sub>10</sub> increase in serum HBV DNA from nadir. However, breakthrough infection also can be a result of noncompliance.

Genotypic resistance can be detected in 14% to 32% of HBeAg-positive patients after 1 year of lamivudine treatment (467–469) and increases with the duration of treatment to 60% to 70% after 5 years of treatment (470,472,473). Retrospective analysis of 998 HBeAg-positive patients who received a median of 4 years of lamivudine treatment identified Asian ethnicity, high pretreatment serum HBV DNA levels, male gender, and longer duration of lamivudine treatment as factors that correlated with the development of lamivudine resistance (473). One study found that a high level of residual virus: Serum HBV DNA greater than 10<sup>3</sup> copies/mL after 6 months of treatment was associated with a higher rate of lamivudine resistance: 63% versus 13% (495). The rates of lamivudine resistance in patients treated for HBeAg-negative chronic hepatitis B appear to be more variable, 0% to 27% at 1 year and 10% to 56% at 2 years (475–477,496).

The clinical course of patients with lamivudine resistant mutants is variable. In vitro studies showed that M204V/I mutation decreases replication fitness of HBV (497,498) but compensatory mutations selected during continued treatment, such as L180M, can restore replication fitness. Therefore, serum HBV DNA levels tend to be lower than baseline when breakthrough infection is first diagnosed. However, over time, serum HBV DNA levels increase and may become higher than pretreatment values. Virologic breakthrough is usually followed by biochemical breakthrough (480). In some patients emergence of lamivudine resistant mutants may be accompanied by acute exacerbations of liver disease (499,500) but exacerbations associated with emergence of lamivudine resistance may also lead to HBeAg seroconversion (470,501). The frequency of hepatitis flares increases with the duration of lamivudine resistance, from 43% in year 1 to greater than 80% after 3 years (473). The occurrence of icteric flares and hepatic decompensation is rare in young precirrhotic patients (6% after 4 years of lamivudine resistance), but more common and may be fatal in older patients and those with advanced fibrosis or cirrhosis. Withdrawal of treatment in patients who have developed lamivudine resistance has been reported to be associated with rapid outgrowth of wild-type virus and flares in liver disease (502,503). However, two studies in Asia found that hepatitis flares and decompensation were similar among patients with lamivudine breakthrough, who stopped or continued lamivudine treatment (504,505).

## Long-term outcome of lamivudine-treated patients

Follow-up of patients receiving continued lamivudine treatment showed that the rates of maintained virologic and biochemical response decrease with time due to selection of drug-resistant mutants. As a group, liver histology after 3 years of treatment is improved compared to baseline but histologic benefit after the first year of treatment is negated among patients with breakthrough infection (479,486). Despite increasing rates of breakthrough infection, two studies with median follow-up of 2 to 4 years reported that lamivudine treatment decreased the overall rate of hepatic decompensation as well as liver-related mortality (506,507).

## Adverse events

In general, lamivudine is very well tolerated. Various adverse events including a mild (two- to threefold) increase in ALT level have been reported in patients receiving lamivudine, but these events occurred with the same frequency among the controls.

## Dose regimen

The recommended dose for adults with normal renal function (creatinine clearance >50 mL/minute) and no HIV infection is 100 mg daily PO. Dose reduction is necessary for patients with renal insufficiency. Patients with HIV coinfection should be treated with 150 mg b.i.d. doses in addition to other antiretroviral therapies.

## Hepatitis B e antigen–positive patients

The end point of treatment for HBeAg-positive patients is HBeAg seroconversion. In general, lamivudine should be administered for a minimum of 6 months after confirmed HBeAg seroconversion. With the availability of newer treatments with lower risk of drug resistance, whether lamivudine should be continued in patients who have been on treatment for more than a year and have not achieved HBeAg seroconversion nor developed breakthrough infection or switched to new therapies is unclear. For patients who have breakthrough infection due to drug resistance, the vast majority should receive rescue therapy with antiviral agents that are effective against lamivudine-resistant HBV mutants. A minority of patients who do not have underlying cirrhosis or immunosuppression may consider stopping treatment, particularly if the indications for initial treatment were weak.

Acute exacerbations of hepatitis with or without hepatic decompensation may occur after discontinuation of lamivudine therapy. Exacerbations may occur even in patients who have developed HBeAg seroconversion and may be up to 1 year (median 4 months) after

cessation of treatment (501,502). Therefore, all patients should be closely monitored after treatment is discontinued. Reinstitution of lamivudine treatment is usually effective in controlling the exacerbations in patients who have not developed breakthrough infection, and may result in subsequent HBeAg seroconversion but the benefits of retreatment are usually transient in patients with breakthrough infection as resistant mutants are quickly selected when lamivudine treatment is resumed (496,503).

#### *Hepatitis B e antigen–negative patients*

The end-point of treatment for HBeAg-negative chronic hepatitis B is unknown. Post-treatment relapse can occur even in patients with undetectable serum HBV DNA by PCR assay. Because of the high rate (approximately 90%) of relapse in patients who responded after 1 year of treatment, longer duration of treatment is recommended. However, the criteria for discontinuation of treatment and the optimal duration of therapy have not been established.

### FAMCICLOVIR

Famciclovir is the oral prodrug of penciclovir, an acyclic deoxyguanosine analog. Penciclovir is phosphorylated to its triphosphate (PCV-TP) which competes with 2'-deoxyguanosine-5'-triphosphate (dGTP) for incorporation into the nascent HBV DNA chains. In addition, PCV-TP may compete with dGTP for the priming of reverse transcription (synthesis of the first DNA strand) (508).

A phase III clinical trial of 417 patients with HBeAg-positive chronic hepatitis B found that the median decrease in histologic activity index among the patients who received famciclovir 500 mg t.i.d., famciclovir 1,500 mg daily or placebo were 1.5, 1, and 0, respectively. Compared to controls, a higher rate of HBeAg seroconversion was observed among the patients who received famciclovir 500 mg t.i.d. (9% vs. 3%), but not in the group who received famciclovir 1,500 mg daily (509).

Drug resistant mutants have also been reported in patients who have been on long-term famciclovir treatment (510). In view of the low efficacy, need for administration thrice daily, and potential for cross-resistance with lamivudine, it is unlikely that famciclovir will have a major role in the treatment of chronic hepatitis B.

### ADEFOVIR DIPIVOXIL (HEPSERA, BIS-POM PMEA)

Adefovir is a nucleotide analog of adenosine monophosphate. It can inhibit reverse transcriptase as well as DNA polymerase activity (511). In vitro and clinical studies

showed that it is effective in suppressing wild type as well as lamivudine-resistant HBV (512).

### Efficacy

1. *HBeAg-positive chronic hepatitis*. A phase III clinical trial included 515 patients with compensated liver disease randomized to receive two doses of adefovir (30 or 10 mg daily) or placebo (Table 29.12) (513). After 48 weeks of treatment, histologic improvement was observed in 59%, 53%, and 25%, respectively. Median decrease in serum HBV DNA level was  $-4.8$ ,  $-3.5$ , and  $-0.6$   $\log_{10}$  copies/mL, while the proportion of patients with undetectable HBV DNA by PCR assay was 39%, 21%, and 0, respectively. Normalization of ALT was observed in 55%, 48%, and 16% whereas HBeAg seroconversion occurred in 14%, 12%, and 6%, respectively. All assessments of response showed a statistical difference between the two treatment groups and the placebo group, and a trend indicating superiority of the higher dose (30 mg) group. However, the 30-mg dose was associated with nephrotoxicity albeit less frequently than the doses (60 and 120 mg) initially used for HIV infection.
2. *HBeAg-negative chronic hepatitis*. A phase III clinical trial included 185 patients with compensated liver disease randomized to receive adefovir 10 mg daily or placebo (514). After 48 weeks of treatment, patients who received adefovir were more likely to have improvement in liver histology (77% vs. 33%), undetectable HBV DNA by PCR assay (51% vs. 0), and normalization of ALT (72% vs. 29%). A follow-up report of 70 patients found that after 144 weeks of continued adefovir treatment, median HBV DNA decrease compared to baseline was 3.6  $\log_{10}$  copies/mL, 79% patients had undetectable HBV DNA by PCR, and 69% patients had normalization of ALT (515). Two patients lost HBeAg. These data indicate that response can be maintained in 70% to 80% patients but as many as 20% to 30% failed to achieve virologic or biochemical response after 3 years of continued adefovir treatment.
3. *Decompensated cirrhosis/liver transplantation*. Data on adefovir as de novo therapy in patients with decompensated cirrhosis or liver transplantation are not available. It is likely that the efficacy will be similar to that in patients who received adefovir as salvage therapy for lamivudine-resistant HBV.
4. *Lamivudine-resistant HBV*. Clinical trials confirmed that adefovir is effective in suppressing lamivudine-resistant HBV (516–519). One study that included 19 patients randomized to receive adefovir monotherapy found that antiviral efficacy of adefovir alone was comparable to that of combination therapy of adefovir and lamivudine (516). However, hepatitis

flares were more common during the transition period. In addition, recent data showed that sequential monotherapy increases the risk of adefovir resistance (520). In patients with decompensated liver disease, addition of adefovir has been shown to result in clinical improvement, reduction in risk of recurrent hepatitis B post-liver transplant, and possibly increased survival (518,519). One study included 128 pre- and 196 post-transplant patients (518). Among the patients who received 48 weeks treatment, 81% of the pre- and 34% of the post-transplant patients had undetectable HBV DNA by PCR assay, and 76% and 49%, respectively had normalization of ALT. Child-Turcotte-Pugh score improved in more than 90% of patients in both groups, and 1-year survival was 84% for the pre- and 93% for the post-transplant patients. Adefovir when added to existing HIV treatment regimen which included lamivudine 150 mg b.i.d. has also been shown to be effective in decreasing serum HBV DNA levels in patients with HIV and HBV coinfection and lamivudine-resistant HBV (521).

### Predictors of response

Detailed analyses of predictors of response had not been performed. Retrospective analyses of data from the two phase III clinical trials showed that HBV DNA reduction was comparable across the four major HBV genotypes (A to D) (362), but an association between adefovir-related HBeAg seroconversion or durability of response and HBV genotypes could not be analyzed because of the small number of responders. HBV DNA reduction was also similar among Asians and Caucasians. Limited data suggest that HBeAg-positive patients with high pretreatment ALT were more likely to undergo HBeAg seroconversion and to have histologic improvement.

### Durability of response and long-term outcome of adefovir-treated patients

#### *Hepatitis B e antigen–positive chronic hepatitis*

Analyses of long-term data were hampered by errors in treatment assignment during the second year of the phase III trial. HBeAg seroconversion rates increased during the second and third years of treatment but the exact figures were unclear. Durability of HBeAg seroconversion was examined in 76 patients who had achieved HBeAg seroconversion after a median of 80 (30–193) weeks adefovir treatment, and had been followed for a median of 52 (5–125) weeks off treatment. HBeAg seroconversion was maintained in 69 (92%) patients (522). The seemingly higher rate of durability of adefovir-related HBeAg seroconversion compared to lamivudine may be related to a longer duration of

treatment (80 vs. <52 weeks) and more importantly, a longer duration of consolidation treatment.

#### *Hepatitis B e antigen–negative chronic hepatitis*

A follow-up report found that almost all 40 patients who received adefovir in year 1 of the phase III trial, who were randomized to placebo in year 2 relapsed. At week 96 (48 weeks after stopping adefovir), only 8% patients had undetectable HBV DNA by PCR assay and 32% had normal ALT (515). These data indicate that longer duration (>48 weeks) of treatment is necessary to achieve sustained response but the optimal duration remains to be determined. Most patients maintained their response when adefovir was continued for 3 years, but there was minimal incremental response during years 2 and 3.

#### *Hepatitis B s antigen loss*

A retrospective analysis of 578 patients who received adefovir monotherapy found that nine (1.6%) patients had HBsAg seroconversion after a median of 73 weeks (523), indicating that HBsAg loss is a rare event.

### Dose regimen

The approved dose of adefovir is 10 mg daily PO. Although higher doses appeared to have more potent antiviral effect, concerns for nephrotoxicity limit their use in clinical practice. Adefovir at the approved dose of 10 mg daily is ineffective in inhibiting HIV replication.

For HBeAg-positive patients, treatment should be administered for an additional 6 months after HBeAg seroconversion is achieved. For HBeAg-negative patients, long-term treatment is needed but the optimal duration of treatment has not been determined. Patients must be closely monitored for relapse when treatment is stopped. Because of the weak antiviral effects of the 10-mg dose, 25% to 50% patients will have a suboptimal virologic response with less than 3.5 log<sub>10</sub> reduction in HBV DNA after 48 weeks of treatment (524); whether these patients should be switched to other more potent antiviral agents such as entecavir and when the switch should occur have not been examined.

### Adverse events

Adefovir is in general well tolerated with similar side effect profile as placebo in the phase III clinical trials in patients with compensated liver disease. The most worrisome adverse event is nephrotoxicity—increase in serum creatinine and/or renal tubular defects manifested as hypophosphatemia and Fanconi's syndrome, which was common at high doses (60 to 120 mg daily). A reproducible increase in serum creatinine by greater than or equal to 0.5 mg/dL has not been reported in

patients who received 10 mg doses after 48 weeks and in 6% after 192 weeks of treatment (513–515,525). In all cases, the increase in serum creatinine was reversible but a few patients had to be withdrawn from treatment. Nephrotoxicity was more common in patients with decompensated cirrhosis and in those who had undergone liver transplantation; a reproducible increase in serum creatinine by greater than or equal to 0.5 mg/dL was observed in 15% to 22% of these patients after 48 weeks of 10 mg doses (518). Whether the high frequency of nephrotoxicity in this setting is a direct result of adefovir, concomitant nephrotoxic medications, pre-existing renal dysfunction or hepatorenal syndrome or a combination of these factors is unclear. Renal function should be monitored in all patients receiving adefovir and dosing intervals adjusted in patients with estimated creatinine clearance less than 50 mL/minute.

### Adefovir resistance

Resistance occurs at a slower rate during adefovir treatment compared to lamivudine. Several mechanisms have been suggested that might contribute to the low rate of resistance to adefovir. The adefovir molecule is structurally similar to 2'-deoxyadenosine 5'-triphosphate (dATP), which limits steric discrimination by HBV polymerase. The phosphonate bond in adefovir may be less susceptible to chain terminator removal once the molecule is incorporated into viral DNA. In addition, its flexible molecular structure permits it to bind HBV polymerase even in the presence of minor alterations in the nucleotide binding pocket. Resistance to adefovir has not been detected in clinical trials of patients who received 48 weeks of treatment (526). However, novel mutations conferring resistance to adefovir (asparagine to threonine substitution N236T and alanine to valine or threonine substitution A181V/T) have been described (527,528). Aggregate data from five studies including three studies using combination of adefovir and lamivudine in patients with lamivudine-resistant HBV estimated the cumulative rate of resistance to be 15% by 192 weeks (529). Another study of adefovir monotherapy in 67 HBeAg-negative patients found that the cumulative rate of resistance was 0%, 3%, 11%, and 18% at week 48, 96, 144, and 192 respectively (525).

In vitro studies showed that adefovir-resistant mutations decrease susceptibility by 3 to 15-fold (528,530). Nevertheless, clinical studies found that viral rebound, hepatitis flares and even hepatic decompensation can occur (531). Therefore, all patients receiving long-term adefovir should be closely monitored for resistance. Risk factors for adefovir resistance that have been identified include suboptimal virus suppression and sequential monotherapy. A pooled study of 467 patients with lamivudine-resistant HBV found that resistance

to adefovir occurred only in patients who stopped lamivudine (520). Sequential treatment with lamivudine followed by adefovir had also been reported to select dual-resistant HBV mutants.

In vitro and human studies showed that adefovir-resistant HBV mutants are susceptible to lamivudine and entecavir (527,528,531). However, the duration of benefit is unknown, and may be short lived in patients with earlier lamivudine resistance.

### ENTECAVIR (BARACLUDE)

Entecavir is an orally administered cyclopentyl guanosine analog. In vitro studies as well as studies in woodchucks showed that it has potent antiviral effects against HBV. One study found that long-term treatment of woodchucks chronically infected with WHV infection resulted in decreased incidence of HCC and increased survival (334). In vitro studies showed that entecavir is effective in suppressing lamivudine-resistant HBV but susceptibility is reduced compared to wild type HBV (414). Entecavir has also been found to be effective in suppressing adefovir-resistant HBV in in vitro studies. Clinical trials confirmed the efficacy of entecavir in humans (532).

### Efficacy

1. HBeAg-positive patients. In a phase III clinical trial 715 patients with compensated liver disease were randomized to receive entecavir 0.5 mg or lamivudine 100 mg daily (Table 29.12). At week 48, entecavir resulted in statistically higher rate of histologic (72% vs. 62%), virologic (HBV DNA undetectable by PCR) (67% vs. 36%) and biochemical (68% vs. 60%) responses (533) compared to lamivudine. However, despite more potent viral suppression (6.9 vs. 5.4 log<sub>10</sub> copies/mL), HBeAg seroconversion rates were similar in the two groups: 21% versus 18%.
2. HBeAg-negative patients. In a phase III clinical trial 648 patients with compensated liver disease were randomized to receive entecavir 0.5 mg or lamivudine 100 mg daily. At week 48, entecavir resulted in statistically higher rate of histologic (70% vs. 61%), virologic (90% vs. 72%) and biochemical (78% vs. 71%) responses compared to lamivudine (534).
3. Lamivudine-refractory HBV. In one study 286 patients, who had persistent viremia while on lamivudine with or without confirmed lamivudine-resistant mutations, were randomized to receive entecavir 1.0 g or lamivudine 100 mg daily. At week 48, entecavir resulted in statistically higher rate of histologic (55% vs. 28%), virologic (21% vs. 1%) and biochemical (75% vs. 23%) responses compared to lamivudine (535).

## Predictors of response

Predictors of response have not been examined in detail. Entecavir appears to be equally effective in decreasing serum HBV DNA level and in inducing histologic improvement in Asians and Whites, across HBV genotypes A-D and a wide range of pretreatment HBV DNA and ALT levels. However, HBeAg seroconversion rates are lower in patients with pretreatment ALT less than  $2 \times$  normal.

## Durability of response

Limited data suggest that durability of response to entecavir is similar or better than lamivudine.

## Dose regimen

The approved dose for nucleoside-naïve patients is 0.5 mg daily PO and for lamivudine-refractory patients is 1.0 mg daily PO. Doses should be adjusted for patients with estimated creatinine clearance less than 50 mL/minute.

## Adverse events

Entecavir had a similar safety profile including on-treatment ALT flares as lamivudine in clinical trials. Studies in mice found an increased risk of lung adenomas (at exposures 3 to 40 times those in humans) (532). In addition, HCC were increased in male mice while brain gliomas were increased in male and female rats. Whether these observations have relevance to humans is unclear. To date, no difference in incidence of HCC or other neoplasms has been observed between patients who received entecavir versus lamivudine but the duration of follow-up is limited.

## Entecavir resistance

No resistance was observed after 48 weeks of treatment in the two phase III clinical trials of nucleoside-naïve patients, although resistance was detected in 7% of patients by week 48 in the trial of lamivudine refractory patients (533–535). Mutations associated with entecavir resistance have been localized to rtI169, rtT184, rtS202, and rtM250 (536). These mutations on their own have minimal effect on susceptibility to entecavir, but when present with lamivudine-resistant mutations, decrease susceptibility to entecavir by greater than 1,000-fold (537).

## EMTRICITABINE (EMTRIVA, FTC)

Emtricitabine is a potent inhibitor of HIV and HBV replication. FTC has been approved for HIV treatment as Emtriva (FTC only) and as Truvada (in combination with tenofovir as a single pill). Because of its structural

similarity with lamivudine (3TC), treatment with FTC selects for the same resistant mutants.

A phase II trial that included 98 patients (77 HBeAg positive) with chronic hepatitis B randomized to receive varying doses of FTC found that 200 mg had the maximum effect on viral suppression (538).

In another study, 248 patients (63% were HBeAg positive) were randomized to receive FTC 200 mg daily or placebo in a 2:1 ratio (539). At week 48, FTC resulted in a significantly higher rate of histologic (62% vs. 25%), virologic (undetectable HBV DNA by PCR assay) (54% vs. 2%) and biochemical (65% vs. 25%) responses but HBeAg seroconversion rates were identical—12% in the two groups. FTC-resistant mutations were detected in 13% patients who received FTC. The high rate of drug resistance and the lack of improvement on HBeAg seroconversion indicate that FTC on its own has no role in the rapidly expanding treatment armamentarium for hepatitis B.

## TENOFOVIR (VIREAD)

Tenofovir disoproxil fumarate is a nucleotide analog that has been approved for the treatment of HIV infection as Viread (tenofovir only) or Truvada (tenofovir + emtricitabine as a single pill). Tenofovir is structurally similar to adefovir. In vitro studies showed that tenofovir and adefovir are equipotent. Because tenofovir appears to be less nephrotoxic, the approved dose is much higher than that of adefovir, 300 mg versus 10 mg daily. This may explain why tenofovir has more potent antiviral effects in clinical studies. Tenofovir has not been approved for treatment of hepatitis B; clinical studies designed to evaluate its safety and efficacy in patients with chronic hepatitis B, particularly those with HBV mono-infection and no earlier lamivudine treatment are under way. Currently, most of the data on tenofovir are based on studies of patients with HBV and HIV coinfection, who have lamivudine-resistant HBV.

Retrospective analysis of two large multicenter HIV trials that included subsets of patients with chronic hepatitis B ( $n = 23$ ) demonstrated that tenofovir was associated with a significant reduction in HBV DNA levels both in patients who had not previously received anti-HIV therapy, as well as those who had already been exposed to anti-HIV therapy (540). Several pilot studies and case series confirmed that tenofovir is effective in decreasing serum HBV DNA levels in patients (with and without HIV coinfection) with lamivudine-resistant HBV (541–545).

In a study of 53 patients with lamivudine-resistant HBV, tenofovir led to a greater reduction in serum HBV DNA levels than adefovir (546). Although the study was not randomized, and there was heterogeneity in patient characteristics and treatment regimen (some but not all continued lamivudine), the finding that

tenofovir is superior to adefovir in clinical practice is not surprising due to the 30-fold difference in dose. Viral rebound has also been reported when patients were switched from tenofovir to adefovir (547).

Tenofovir is less nephrotoxic than adefovir. However, tenofovir has been occasionally reported to cause Fanconi's syndrome and renal insufficiency (548–550). Almost all of these adverse events occurred in patients with HIV coinfection receiving antiretroviral therapy. The likelihood of tenofovir-associated nephrotoxicity in patients with HBV monoinfection is unclear. Nevertheless, all patients receiving long-term tenofovir should be closely monitored, particularly those with baseline impaired renal function and those with decompensated liver disease.

### L-DEOXYTHYMIDINE (LdT, Telbivudine) AND VAL-DEOXYCYTOSINE (VAL-LdC)

L-deoxythymidine (LdT) and val-deoxycytosine (val-LdC) are nucleoside analogs with potent antiviral effects against HBV. However, they select for the same mutations as lamivudine, FTC and clevudine.

A phase II trial included 104 HBeAg-positive patients who were randomized to receive LdT (400 or 600 mg daily) alone or in combination with lamivudine 100 mg daily, or lamivudine alone (551). The two LdT monotherapy groups had higher rates of virologic (61% vs. 32%) and biochemical (86% vs. 63%) responses compared to the lamivudine group. HBeAg seroconversion rates were similar in the two groups: 31% versus 22%. Combination of LdT and lamivudine did not confer any benefit; in fact the combination group appeared to fare worse than the LdT monotherapy group. Mutations in the YMDD motif were detected in 4.5%, 9.8% and 15.8% of patients who received LdT alone, LdT and lamivudine, and lamivudine alone, respectively. A follow-up report on 90 patients who continued treatment for 96 weeks showed that LdT continued to be superior to lamivudine in virologic and biochemical responses but the difference in HBeAg seroconversion rates was not statistically significant (38% vs. 21%) (552). Mutations in the YMDD motif were detected in six additional patients in the LdT alone group during the second year of therapy, resulting in a cumulative resistance rate of 18% at the end of year 2. Phase III clinical trial is ongoing. Given the availability of newer antiviral agents with lower risk of drug resistance, the role of LdT in hepatitis B treatment is limited.

Val-LdC is a well absorbed oral prodrug of LdC. Phase I/II clinical trials showed that it is effective in suppressing HBV replication (553). In vitro and woodchuck studies found that val-LdC and LdT have additive antiviral effects.

### CLEVDINE (LFMAU, 2'-FLUORO-5-METHYL-L-ARABINOFURANOSYL URACIL)

Clevudine is a pyrimidine nucleoside analog that is effective in inhibiting HBV replication in vitro and in animal models. Phase I/II clinical trials confirmed that a 4-week course of clevudine can decrease serum HBV DNA levels by 2 to 3 log<sub>10</sub> copies/mL and at high doses, HBV DNA levels remained suppressed for up to 24 weeks after stopping treatment (554,555). Clinical trials are ongoing to determine the optimal dose regimen of clevudine and to determine if sustained response can be achieved after a longer (24 week) course of treatment. One drawback of clevudine is that it selects for the same mutations as lamivudine and FTC.

### COMBINATION THERAPIES

Monotherapy with a single antiviral agent or IFN- $\alpha$  is unlikely to be sufficient for the eradication of HBV infection in most patients with chronic hepatitis B. Combination therapies have been proven to be more effective than monotherapy in the treatment of HIV and HCV infection. The potential advantages of combination therapies are additive or synergistic antiviral effects, and diminished or delayed resistance. The potential disadvantages of combination therapies are added costs, increased toxicity, and drug interactions. Various combination therapies have been evaluated; to date, none of the combination therapies has been proven to be superior to monotherapy in inducing a higher rate of sustained response or in decreasing the rate of drug resistance. Nonetheless, continued effort should be expended to develop an optimal combination therapy for hepatitis B.

#### Standard or pegylated interferon- $\alpha$ and lamivudine

The combination of IFN- $\alpha$  and lamivudine seems logical because monotherapy with each agent is effective, and IFN- $\alpha$  and lamivudine have different mechanisms of action.

##### *Treatment-naïve patients*

Five large trials (one using standard IFN- $\alpha$  and four using pegIFN- $\alpha$ , 4 in HBeAg-positive patients and one in HBeAg-negative patients) were conducted comparing combination of IFN- $\alpha$  and lamivudine to lamivudine alone and/or IFN- $\alpha$  alone (359,460,461,463,469). All studies found that combination therapy had greater on-treatment virus suppression but there was no difference in sustained off-treatment virologic response compared to IFN- $\alpha$  alone. All studies showed that



combination therapy resulted in higher rates of sustained off-treatment response compared to lamivudine alone. Although combination therapy was associated with lower rates of lamivudine resistance compared to lamivudine monotherapy, a low rate of lamivudine resistance was encountered compared to none in patients who received IFN- $\alpha$  alone.

#### *Interferon- $\alpha$ nonresponders*

Combination therapy of standard IFN- $\alpha$  and lamivudine is not more effective than lamivudine alone in the retreatment of IFN- $\alpha$  nonresponders (481).

### **Lamivudine and adefovir**

#### *Nucleosid(t)e naïve patients*

One trial included 115 patients randomized to receive a combination of lamivudine and adefovir or lamivudine alone. At week 52, there was no difference in HBV DNA suppression, ALT normalization or HBeAg loss (556). However, combination therapy was associated with a lower rate of detection of lamivudine-resistant mutations: 20% versus 2% suggesting that the benefit of combination therapy may be observed with continued treatment.

#### *Patients with lamivudine-resistant hepatitis B virus*

One small trial in patients with compensated liver disease showed that combination of adefovir and lamivudine was not superior to adefovir alone in decreasing serum HBV DNA levels (516). However, hepatitis flares were less frequent during the transition period in the combination therapy group. Recent data suggest that continuation of lamivudine reduces the risk of resistance to adefovir (520). There is therefore increasing evidence to support the conclusion that combination of adefovir and lamivudine is superior to adefovir monotherapy for patients with lamivudine-resistant HBV.

### **Lamivudine and telbivudine**

One trial conducted in nucleoside naïve HBeAg-positive patients demonstrated that combination of lamivudine and telbivudine had no advantage over telbivudine alone (551). In fact, the combination group showed a trend toward an inferior result in all parameters: Virus suppression, ALT normalization, HBeAg seroconversion, and mutations in the YMDD motif. These data suggest that lamivudine and telbivudine, both being L-nucleosides, may antagonize each other by competing for the same binding site on the HBV reverse transcriptase.

### **Adefovir and Emtricitabine**

A pilot trial on 30 HBeAg-positive patients found that patients randomized to receive combination of adefovir and emtricitabine had greater decrease in serum HBV DNA levels compared to those who received adefovir alone (557). Whether this was related to an additive or synergistic effect of these two compounds or an unusually poor response in the adefovir monotherapy group (2 to 3 log<sub>10</sub> reduction in serum HBV DNA after 1 year of therapy) is unclear.

## **NOVEL ANTIVIRAL APPROACHES**

Several innovative antiviral approaches have been evaluated in *in vitro* as well as in animal models of chronic hepatitis.

### **Selective targeting of antiviral agents to the liver**

Conjugation of antiviral agents to ligands that are selectively taken up by the liver may permit these agents to be used in lower doses with decreased systemic adverse effects. Several systems of selective delivery have been evaluated including conjugation to lactosaminated human serum albumin, liposome encapsulation, and incorporation into recombinant chylomicrons (558,559).

### **Antisense approaches**

Transcription and translation of HBV DNA and HBV RNA can be prevented by antisense molecules or ribozymes that are complementary to the DNA or RNA templates (560,561). These molecules can be delivered by the administration of preformed molecules or vector DNA. The advantage of this approach is that specific targets can be precisely selected. In addition, the risk of drug-resistant mutants can be reduced by targeting multiple sites in the viral DNA or RNA or by targeting regulatory sequences that would not tolerate mutations. *In vitro* studies have confirmed that this approach is feasible. The major impediments to the clinical use of antisense treatment include rapid degradation of the antisense molecules by nucleases *in vivo*, lack of an efficient delivery system into the target cells, and hindrance of access to target DNA or RNA sequences by secondary structure.

### **Short interfering RNA**

RNA interference (RNAi) is a recently discovered cellular mechanism that detects and destroys double-stranded RNA and seems to play a role in the cell's antiviral defense system (562). Short interfering RNA (siRNA) molecules are approximately 21-nucleotide,

double-stranded RNA intermediates of the RNAi mechanism that guides a unique RNA-induced silencing complex to target RNA, leading to its subsequent degradation. Administration of synthetic siRNAs that use the endogenous cellular mechanism to downregulate the expression of HBV genes is a potential novel approach to treatment of hepatitis B. Several investigators have demonstrated the efficacy of siRNA in inhibiting HBV expression in cell culture systems and more recently in animal models (563–567). Nevertheless, major hurdles in delivering adequate amounts of siRNA to the target cells need to be overcome before this approach can be tested in humans.

## IMMUNOMODULATORY THERAPY

*Nonspecific immunomodulation* is largely ineffective in clearing HBV infection.

### Thymosin

Thymic-derived peptides can stimulate T cell function. Thymosin is well tolerated but data on efficacy are conflicting (568–571). A meta-analysis that included five controlled trials with a total of 353 patients concluded that patients treated with thymosin were significantly more likely than controls to have a virologic response (572). The maximal rate of response was not seen until 12 months after discontinuing therapy (odds ratio 1.67, 95% CI, 0.83 to 3.37). Thymosin is approved for the treatment of hepatitis B in some countries, mainly in Asia.

### Hepatitis B virus–specific immunomodulation

In the past few years, several HBV-specific immunomodulatory therapies have been developed, some of which have shown promise.

#### *S* and *pre-S* antigen vaccines

Several uncontrolled trials reported that vaccines with HBV *S* with or without *pre-S* antigens used for prevention of HBV infection were effective in inducing anti-HBs response and in decreasing serum HBV DNA levels in patients with chronic hepatitis B (573,574). These data need to be confirmed in controlled clinical trials.

#### *Deoxyribonucleic acid* vaccination

Unlike peptide vaccines, vaccination with plasmid DNA that express viral proteins *in situ* can stimulate not only B cell but also T cell (both helper and cytotoxic) response. In addition, DNA vaccines lead to more prolonged expression of viral proteins (575). Studies in mice have shown that vaccination with plasmids

that contain HBV surface gene can induce anti-HBs response. DNA vaccination has been demonstrated to decrease the production of HBsAg in transgenic mice that express the HBV surface gene (576). One pilot study reported that DNA vaccination was also effective in activating T cell response and in decreasing serum HBV DNA levels in patients with chronic HBV infection (577).

#### *T* cell vaccines

Patients with chronic HBV infection have been demonstrated to have impaired cytotoxic T lymphocyte response to HBV antigens, resulting in ineffective virus clearance. *In vitro* and animal (transgenic mice) studies show that CTL response to HBcAg can be induced by repeated exposure to peptides that correspond to major HBcAg epitopes. Moreover, administration of HBsAg primed CTLs to transgenic mice that express HBV surface gene can result in decreased transcription of viral RNA and translation of viral antigens with minimal cell damage (578). These data suggest that vaccination with synthetic peptides that stimulate CTL response to HBV antigens can induce viral clearance without causing massive hepatocyte damage. One Phase II study showed that CTL response can be stimulated in patients with chronic HBV infection, who were inoculated with a vaccine that contained an HLA-restricted HBcAg CTL epitope but the antiviral effect was weak (579).

## RECOMMENDATIONS FOR THE TREATMENT OF CHRONIC HEPATITIS B

Current therapy of chronic hepatitis B has limited long-term efficacy. Therefore a careful balance of patient's age, severity of liver disease, likelihood of response, and potential adverse events and complications is needed before treatment is initiated. Except for patients with decompensated cirrhosis, where IFN- $\alpha$  or pegIFN- $\alpha$  are contraindicated, all approved treatments should be considered. For patients who require long-term therapy such as patients with HBeAg-negative chronic hepatitis and those with cirrhosis, lamivudine is not an optimal therapy because of the high risk of drug resistance. For patients with severe acute exacerbation lamivudine or entecavir is preferred as initial therapy because of the slow and sometimes inconsistent antiviral effects of adefovir. The pros and cons of the approved therapies are listed in Table 29.13. Table 29.14 summarizes current recommendations for the treatment of chronic hepatitis B (580–582).

Treatment of hepatitis B has evolved at a rapid pace in the past 10 years. With the availability of many new treatment modalities, it is now possible to contemplate combination therapy for hepatitis B. The question is which is the right combination? As our understanding

**TABLE 29.13. PROS AND CONS OF APPROVED TREATMENTS OF HEPATITIS B**

	Standard/pegylated IFN	Lamivudine	Adefovir	Entecavir
Route of administration	Parenteral	Oral	Oral	Oral
Duration of treatment	Finite, ~12 m	Indefinite, y	Indefinite, y	Indefinite, y
Viral suppression	Weakest, but has immunomodulatory effects	Potent	Weak, suboptimal response in ~25%	Most potent
Efficacy against lamivudine-resistant HBV	Unclear	NA	Yes	Yes, activity lower than for wild type HBV
Resistant mutations	None identified	15%–30% y 1, 70% y 5	0% y 1, 29% y 5	0% y 1
Side effects	Frequent, may be serious	Negligible	Nephrotoxic, ~5% y 3	Limited safety record carcinogenic in rodents at high doses

IFN, interferon; HBV, hepatitis B virus.

**TABLE 29.14. RECOMMENDED STRATEGIES FOR PATIENTS WITH CHRONIC HEPATITIS B**

HBeAg	HBV DNA <sup>a</sup> > 5 log <sub>10</sub> IU/mL	ALT <sup>a</sup>	Recommended strategy
+	+	≤2 × ULN	<ul style="list-style-type: none"> <li>• Low efficacy of available therapies</li> <li>• Monitor ALT and HBV DNA levels every 3–6 m</li> <li>• Consider treatment if ALT increases to &gt;2 × ULN</li> </ul>
+	+	>2 × ULN	<ul style="list-style-type: none"> <li>• Consider treatment with one of the five approved therapies</li> <li>• Endpoints of treatment: Standard IFN—16 wk PegIFN—48 wk</li> <li>• Oral nucleoside/tide analogs: Lamivudine, adefovir and entecavir, extend therapy for 6 m after HBeAg seroconversion Lamivudine resistance—Adefovir or Entecavir</li> </ul>
–	–	≤2 × ULN	<ul style="list-style-type: none"> <li>• No treatment required</li> <li>• Monitor ALT and HBV DNA levels every 6–12 m</li> </ul>
–	+	>2 × ULN	<ul style="list-style-type: none"> <li>• IFN and pegIFN preferred in young patients because of finite duration of therapy</li> <li>• Adefovir or Entecavir preferred if oral therapy is chosen because of low rates of antiviral resistance with prolonged therapy</li> <li>• Lamivudine only if cost is a significant factor</li> <li>• Goals of treatment: Undetectable HBV DNA by PCR and normal ALT</li> <li>• Duration of treatment: IFN and pegIFN—48 wk Adefovir and Entecavir—&gt;48 wk</li> </ul>
+	+	Cirrhosis	<ul style="list-style-type: none"> <li>• Compensated—Lamivudine or Adefovir or Entecavir</li> <li>• Decompensated—refer for transplant. IFN/pegIFN contraindicated</li> </ul>
+	–	Cirrhosis <sup>b</sup>	<ul style="list-style-type: none"> <li>• Compensated—observe</li> <li>• Decompensated—refer for transplant</li> </ul>

<sup>a</sup>Liver biopsy should be considered for patients with HBV DNA of 4–5 log<sub>10</sub> IU/mL and ALT 1–2 × ULN, or with fluctuating HBV DNA or ALT levels; those with moderate/severe inflammation, and bridging fibrosis or cirrhosis may benefit from treatment.

<sup>b</sup>Treatment may be considered for patients with cirrhosis even if ALT is normal or HBV DNA is 3–5 log<sub>10</sub> IU/mL, especially if decompensated.

HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; ULN, upper limit of normal; IFN, interferon; PCR, polymerase chain reaction.

Modified from Lok A, McMahon BJ. AASLD practice guidelines: chronic hepatitis B

[http://www.aasld.org/netFORUMAAASLD/eweb/docs/chronichep\\_B.pdf](http://www.aasld.org/netFORUMAAASLD/eweb/docs/chronichep_B.pdf). *Hepatology* 2004;39:857–861.

of hepatitis B improves, it is also possible that future therapies will be tailored to the viral (HBV genotype), immunologic (ALT) and clinical characteristics (stage of liver disease) of the patient.

## Hepatitis D

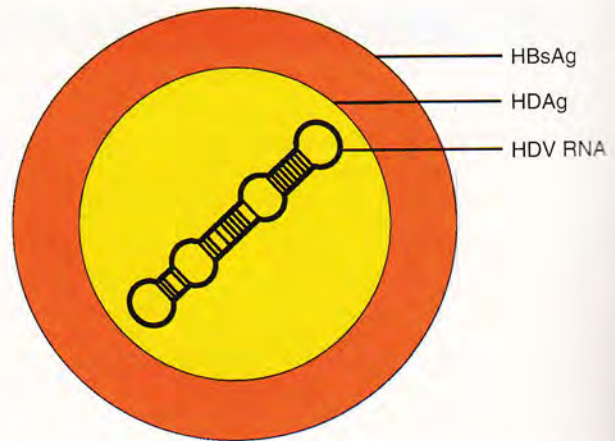
Hepatitis D is caused by a defective virus: The HDV. Although HDV is often referred to as hepatitis delta virus, the term HDV is preferred. HDV can replicate autonomously (583), but the simultaneous presence of HBV is required for complete virion assembly and secretion.

### HEPATITIS D VIRUS STRUCTURE AND REPLICATION

The HDV virion comprises an RNA genome, a single HDV encoded antigen, and a lipoprotein envelope provided by HBV and consisting of the same proteins found in the envelope of the HBV virion. The HDV genome is a small single-stranded circular RNA (1676 to 1683 nucleotides in size) with structural analogies to plant viroids and a high degree of self-complementarity causing the molecule to collapse into a rod-like structure (584). Significant sequence heterogeneity exists among HDV isolates and a classification into three genotypes has been proposed (585), although recent data suggest an even more complex phylogenetic differentiation of HDV into as many as seven clades (586). The only antigen associated with HDV, the hepatitis D antigen (HDAg), is a structural component of the virion: Approximately 70 molecules of HDAg are complexed with the HDV RNA genome to form a ribonucleoprotein structure (587) (Fig. 29.5). Hepatocytes are the only host cells where HDV replicates at very high levels (588) by transcription into a full-length complementary RNA (antigenomic HDV RNA) (584). HDV virion assembly and release is dependent on the simultaneous presence of HBV, which provides the envelope proteins. In the absence of HBV, HDV infection is abortive, unless promptly rescued by HBV (see the following text).

### PATTERNS OF HEPATITIS D VIRUS INFECTION

Due to its dependence upon HBV, HDV infection always occurs in association with HBV infection. The clinical and laboratory findings vary with the type of infection. *Coinfection of HBV and HDV* in an individual susceptible to HBV infection results in an acute hepatitis clinically indistinguishable from classical acute hepatitis B and is usually transient and self-limiting, although a fulminant course was frequently reported among drug



■ **Figure 29.5** Schematic representation of the hepatitis D virus (HDV) virion with extensive intramolecular base-pairing of the HDV RNA genome. HBsAg, hepatitis B surface antigen; HDAg, hepatitis D antigen.

addicts (589). The rate of progression to chronic infection is similar to that observed after HBV monoinfection (590). *HDV superinfection* of a chronic HBsAg carrier may present as a severe acute hepatitis in a previously unrecognized HBV carrier, or as an exacerbation of pre-existing chronic hepatitis B. Progression to persistent HDV infection is typical (591). A third form of infection is a *helper-independent latent HDV infection*, as reported in the woodchuck experimental model (592) and initially thought to occur in the liver transplant setting.

### EPIDEMIOLOGY OF HEPATITIS D VIRUS

Data on HDV epidemiology have mostly been gathered in HBV carriers superinfected with HDV. It was estimated that approximately 5% of the HBV carriers worldwide may be infected with HDV (593). However, substantial changes in HDV epidemiology have occurred in the past 10 years. Improvements in socioeconomic conditions, an increased awareness of the risk of transmitting infectious diseases fostered by acquired immunodeficiency syndrome (AIDS) prevention policy, and aggressive vaccination campaigns against HBV have all contributed to a dramatic decrease in the incidence of HBV infection and the spread of HDV infection, especially in those countries that were previously endemic (594–597).

Although HDV infection is dependent on HBV infection, the geographical distribution of HDV infection does not parallel that of HBV, as areas endemic for HBV may be almost HDV free. The level of HDV endemicity is partly related to the route of transmission. HDV infection is endemic in the Mediterranean basin, where infection tends to occur early in life and is associated with low socioeconomic status. In the Far East, the prevalence of HDV infection among HBV carriers varies