Viral Hepatitis B

16

Dar-In Tai

Key Concepts

- HBV is a highly infectious, small, circular, incomplete double-stranded DNA virus.
- Chronic infection is common when patients are infected in the early stages of life.
- The course of chronic HBV infection is a trilogy, starting with immune tolerance, followed by immune clearance, and finally a residual phase.
- Immune tolerance is preventative against cytokine storm. It could be a survival strategy and is associated with genetic evolution during human migration.
- The severity and duration of liver inflammation determine the pathogenesis of liver cirrhosis and hepatocellular carcinoma.
- Most chronic HBV carriers terminate HBV replication and may achieve delayed HBsAg clearance several decades later.
- Current HBV-specific therapy may suppress viral replication but is unable to clear covalently closed circular DNA in the nucleus. Virologic relapse may reach 50% during the first year after treatment ends.
- New therapeutic strategies and agents are needed for eradication of chronic HBV infection.

16.1 Introduction

Hepatitis B virus (HBV) infection is a cause of chronic liver disease with a long medical history in humans. It was first discovered in 1963 by Baruch Samuel Blumberg (Nobel Prize for Medicine, 1976) when he performed double immunodiffusion assays using sera from aboriginal Australians. Many epidemiological studies soon identified HBV infection as a global chronic disease, with the highest prevalence rates in Asia and Africa. Before vaccination programs in these two continents, the prevalence of hepatitis B surface antigen (HBsAg) was generally above 15%. More than four billion people have been chronically infected with HBV worldwide. The WHO estimated that chronic hepatitis B resulted in 880,000 deaths from cirrhosis and hepatocellular carcinoma (HCC) in 2015.

16.2 HBV Genome

HBV is classified as part of the Hepadnaviridae family, which comprises small, hepatotropic DNA viruses that replicate through reverse transcription [1]. The complete HBV virion is a sphere with a diameter of 40–45 nm. HBV has a 3.2 kbp, circular, incomplete double-stranded DNA genome.

The viral genome encodes four overlapping open reading frames (ORFs: S, C, P, and X). The S ORF encodes the viral surface envelope proteins, which can be structurally and functionally divided into the pre-S1, pre-S2, and S regions. The core, or C, gene contains the pre-core and core regions that are expressed as the hepatitis B e antigen (HBeAg) and hepatitis B c antigen (HBcAg), respectively. The P and X ORFs encode HBV DNA polymerase and the hepatitis B x antigen (HBxAg).

Other important Hepadnaviridae *viruses* within this family are woodchuck hepatitis B *virus*, duck hepatitis *virus*, ground squirrel hepatitis B *virus*, tree squirrel hepatitis B virus, and heron hepatitis B virus.

16.3 HBV Genotypes

HBV co-diverged with modern human migration and evolved into eight genotypes (A–H) in various geographic locations. There is an interaction between human leukocyte antigen (HLA) and HBV genotype [2].

D.-I. Tai (✉)
Liver Research Center, Chang Gung Memorial Hospital, Linkou Medical Center, Taoyuan City, Taiwan

F. Radu-Ionita et al. (eds.), *Liver Diseases*, https://doi.org/10.1007/978-3-030-24432-3_16

The distribution of HBV genotypes is mainly A, D and E in Africa; A and D in Europe; D in south Asia; B and C in East Asian; and F, G and H in America [1]. In general, genotypes A and B have a shorter HBV replication stage and better infection outcome than genotypes C and D.

16.4 HBV Mutation

HBV genome is one of the most variable among DNA viruses. This is mainly related to the error-prone HBV DNA polymerase and its very high virion production. HBV genome mutations occur frequently during the immune clearance stage, vaccination, and anti-HBV therapy. There could be selection of viral strains based on the environmental stress to the virus. This is especially occurred in patients unable to suppress viral replication efficiently. HBV mutants can be found frequently in patients with HCC [3]. Some of these mutants may be related to hepatocarcinogenesis.

16.5 HBV Transmission

HBV primarily infects humans and chimpanzees. It is highly infectious, and most infections occur intra-family or in early childhood [4]. HBV may be transmitted to humans from contaminated food, water, needles, a wound, sexual contact, close contact, or through maternal-fetal exchanges.

HBV enters the host cell by binding with sodium taurocholate co-transporting polypeptide (NTCP) on the cell membrane [5]. NTCP is mainly expressed in hepatocytes, which is one of the main reasons for the hepatotropic effect of HBV. The discovery of this receptor has led to an increased number of studies on HBV replication. NTCP can be transfected into hepatoma cell lines and expressed on the cell membrane. Such NTCP-expressing HCC cell lines may be infected with HBV-contaminated serum. The HBV may replicate in the HCC cells and excrete complete virions into culture media. This replication model greatly supports the screening of therapeutic agents for HBV.

Once it enters the hepatocyte, the relaxed circular DNA (rcDNA) is released from the envelope protein. Through host DNA repair enzymes, the rcDNA is converted to covalently closed circular DNA (cccDNA) in the nucleus. The fundamental role of HBV cccDNA has served as an example for transcription of all viral RNAs, which are required to produce viral genomes and express viral proteins [1].

During active HBV replication, the HBV genome and its products rarely modulate the host's cellular gene transcription [6]. This behavior makes HBV replicate peacefully in hepatocytes without interfering with hepatocyte function and transaminase levels in the immune tolerance phase.

16.6 Acute Infection

Acute hepatitis may develop 2–3 months after an individual is infected with HBV. Serum sickness, urticaria, or arthralgia may occur preceding the elevation of transaminase levels. The symptoms of acute hepatitis vary from no significant symptoms to the development of jaundice and hepatic failure. Most cases of acute infection resolve several months later with the development of anti-hepatitis B surface antigens (anti-HBs). The spontaneously produced anti-HBs may produce life-long protection from HBV infection. In less than 1% of acute hepatitis B patients with jaundice, the disease may progress into fulminant hepatic failure. Disturbed consciousness occurs within 4–8 weeks after the appearance of jaundice [1].

Before the nucleos(t)ide analogues (NA) era, more than 80% of patients with fulminant hepatic failure did not survive. Liver transplantation was the only life-saving therapy. At present, artificial liver supporting system and NA therapy can be offered. NA therapy significantly decreases mortality and necessity for liver transplantation if therapy is started before deep jaundice appears [7]. The artificial liver supporting system could prolong wait times or delay the need for liver transplantation [8].

16.7 Chronic Infection

A patient with persistent HBsAg for more than 6 months is considered a chronic HBsAg carrier. HBV may replicate in hepatocytes without causing direct cytopathy or immune-related cytopathy. This type of chronic infection is related to age and transmission route. In East Asia, maternal to fetus (vertical) or perinatal infection results in an 80–90% persistent infection rate. This rate decreased to around 23% when infection occurred at preschool age, and decreased further to 2.3% when infection occurred at college student age [4]. In African, early termination of HBV replication would prevent maternal-fetal infection. Most chronic HBsAg carriers in Africa contract HBV infection in the early stage of life by horizontal transmission. There is an age-related immune tolerance phase in most mammals, which during infancy may decrease allergy and mortality among neonates. Unfortunately, this mechanism is prone to progress to chronic persistent infection of HBV.

Chronic infection begins with an immune tolerance phase, in which HBV is actively replicated in the liver without causing inflammation. The immune system does not recognize HBsAg or Hepatitis B e antigen (HBeAg), or may recognize them but not elicit a strong immune reaction. Through unknown mechanisms, this initially weak immune response becomes stronger with age. Within two to four decades, an immune clearance reaction will often develop to terminate HBV replication.

Our immune system is carefully orchestrated. Innate immunity, T and B cells, cell-mediated and antibody responses all contribute to HBV clearance.

Once this immune clearance reaction successfully suppresses HBV replication, HBsAg may persist without significant HBV replication in the residual phase. About 50% of HBsAg carriers will ultimately clear HBsAg at age 80 [9]. Those patients unable to clear HBV replication smoothly have an increased risk of chronic hepatitis, liver cirrhosis, and hepatocarcinogenesis [1, 4, 9, 10].

16.8 Genetic Factors Predisposing to Chronic Persistent Infection

Chronic HBV infection and HCC may be clustered in families. This prompted researchers to investigate genetic factors related to chronic HBV infection. In genome-wide association studies conducted with East Asian populations, HLA-DP and -DQ loci were identified to be associated with chronic persistent infection [11, 12]. However, these genetic polymorphisms are present in East Asians only (Fig. 16.1b). Therefore, such genetic risk factors do not play a role in the high prevalence of chronic HBV infection in Africa [13]. Age of infection may underlie the main mechanism for chronic persistent HBV infection in Africans as well as in other continents.

The high prevalence of persistent HBV infection-related HLA-DP and -DQ loci in East Asians probably evolved during human migration. A trend of decreased immune-related gene expression was found for the period shortly after human migration out of Africa (Fig. 16.1a). Higher expression genotypes for IL-28B, interferon lambda 4, complement factor B, and CD40 are more prevalent in Africans than in either Europeans or South Asians [13]. In addition, when human

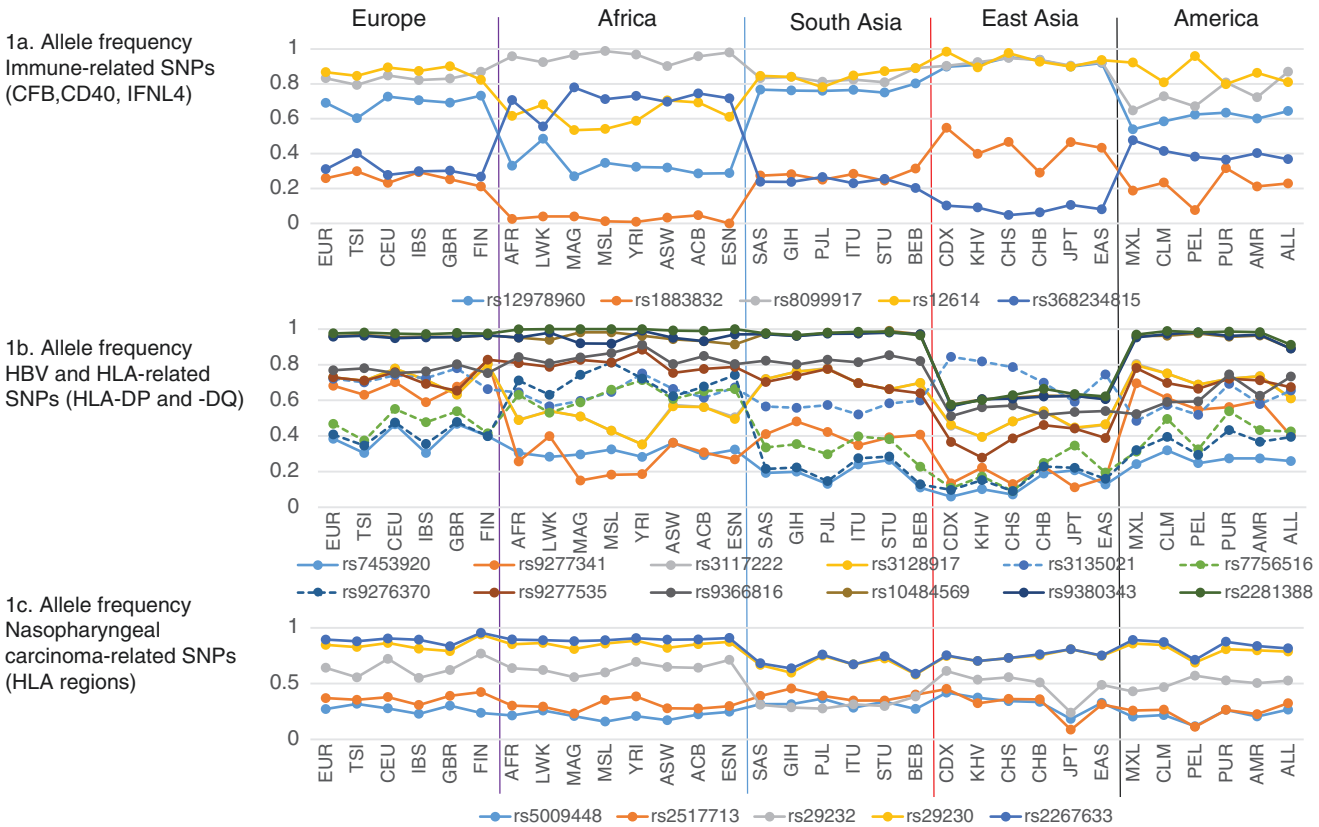

Fig. 16.1 Allele frequency of viral hepatitis- and NPC-related SNPs in different geographic groups. (**a**) Allele frequency of immune-related SNPs (CFB, CD40, and IFNL4). Significant allele type differences were found between African and European populations, and between African and South Asian populations, in all immune-related SNPs. (**b**) Allele frequency of HBV- and HLA-related SNPs (HLA-DP and -DQ). Significant allele differences were found between South and East Asian populations in 8 of 12 HLA-related SNPs, and between African and South Asian populations in 3 of 12 SNPs. (**c**) Allele frequencies of NPC-related SNPs (HLA regions). There was no significant difference among different populations in five NPC-related SNPs. Abbreviations: *ACB* African Ancestry from Barbados in the Caribbean, *AFR* Africa, total, *ALL* global, total, *AMR* America, total, *ASW* African ancestry in Southwest United States, *BEB* Bengali in Bangladesh, *CDX* Chinese Dai in Xishuangbanna, China, *CEU* Utah residents with ancestry from Northern and Western Europe, *CHB* Han Chinese in Beijing, China, *CHS* Han Chinese South, China, *CLM* Colombians in Medellin, Colombia, *EAS* East Asia, total, *ESN* Esan from Nigeria, *EUR* Europe, total, *FIN* Finnish in Finland, *GBR* British from England and Scotland, UK, *GIH* Gujarati Indians in Houston, TX, *IBS* Iberian populations in Spain, *ITU* Indian Telugu in the UK, *JPT* Japanese in Tokyo, Japan, *KHV* Kinh in Hochi Minh city, Vietnam, *LWK* Luhya in Webuye, Kenya, *MAG* Mandinka in Gambia, *MSL* Mende in Sierra Leone, *MXL* Mexican ancestry in Los Angeles, CA, *PEL* Peruvian in Lima, Peru, *PJL* Punjabi in Lahore, Pakistan, *PUR* Puerto Ricans in Puerto Rico, *SAS* South Asia, total, *STU* Sri Lankan Tamil in the UK, *TSI* Toscani in Italy, *YRI* Yoruba in Ibadan, Nigeria

migration reached the Indochina Peninsula, there was a sharp geography change from the flat land in Bangladesh to the mountainous and forested area of Chinese Dai. The latter environment in China and the Indochina Peninsula harbors a great diversity of plants and animals. Regions with higher plant and animal biodiversity often also feature an increased range and abundance of vector- or non-vector-borne diseases. Accordingly, the inhabitants of these areas should be able to handle an increased number of unfamiliar microorganisms. Those subjects who demonstrate direct and strong immune responses may die of cytokine storm in fulminant hepatitis, SARS, influenza, or other infections. Therefore, the persistent HBV infection-related single nucleotide polymorphisms (SNPs) on HLA-DP and -DQ loci could be an adaptive evolutionary response to the local environment.

16.9 Serological Diagnosis of HBV

The HBV genome encodes five proteins. The detection of HBsAg in sera and persistence for 6 months is a strong indication of chronic HBV infection. In the case of chronic hepatitis B under NA therapy, quantitative HBsAg level may have prognostic predict value [14]. The presence of hepatitis B core protein antibody (anti-HBc) is an evidence of nature HBV infection. High titer IgM class anti-HBc can be seen in acute hepatitis B. The presence of HBeAg in patients' serum is an indicator of the immune tolerance phase with active HBV replication. Most patients seropositive for both HBsAg and HBeAg antibody (anti-HBe) are in the residual stage. About 10% of these patients may still have a high serum titer of HBV DNA, which is usually associated with active HBV replication and liver inflammation [15].

The clearance of nuclear cccDNA is an important therapeutic end point. After HBV DNA is carefully digested, serum HBV RNA is assessed as a surrogate marker for cccDNA [16].

16.10 Immune Response

HBV is not directly cytopathic [6]. Most of the inflammation is induced by the immune clearance response. Immune tolerance is a survival strategy [13]. Excess immune response may induce fulminant hepatitis, while weak immune response may result in persistent HBV infection. Chronic HBV infection starts with an immune tolerance phase, followed by the immune clearance phase, and finally progresses to the residual phase.

During the immune tolerance phase, host immune cells are not completely unresponsive to HBV proteins. Selective B cell responses to viral proteins have been documented. Antibodies to HBsAg and HBeAg are generally absent. On the other hand, HBcAg, HBx or HBV DNA polymerase antibodies can be found.

Similar situations are identified in the T cell response. During the immune tolerance phase, HBsAg carriers show PMA (phorbol 12-myristate 13-acetate)/ionomycin-induced cytokine secretion similar to that of healthy controls. However, tumor necrosis factor-alpha and IL-22 levels are higher, and *chemokine* (*C-C* motif) ligand 3 (*CCL3*) levels are lower in HBsAg carriers at the immune tolerance phase than in healthy controls. In addition, programmed cell death protein 1 (PD-1) positive $CD4^+$ and $CD8^+$ T cells are both more frequent in the immune tolerance phase in HBsAg carriers than in healthy controls. IFN-ϒ-producing $CD3^+$ cells induced by HBV-specific peptides are present in HBsAg carriers in immune-tolerant, chronic active hepatitis, and inactive carrier stages. However, a stronger response can be seen in patients with chronic active hepatitis [17, 18].

While adaptive B and T cell responses are important in HBV immune clearance, how to induce a strong adaptive immune response in a patient with immune tolerance is still a mystery. We do not know why HBeAg positive patients in the immune tolerance phase gradually progress to the immune clearance phase. One explanation is that our innate immunity is continuously challenged by microorganisms and environmental substances. The strength of the innate immune response thus increases with age. Toll-like receptor (TLR)-mediated production of anti-inflammatory cytokines (e.g., IL-10) is high in pre-term infants, progressively declines over the first year of life, and is lowest in adults [19]. In contrast, the production of pro-inflammatory cytokines (e.g., IL-1β, TNF-α) gradually increases with age. This age-associated change and other unclear cofactors may orchestrate an immune clearance reaction once a break point is reached.

16.11 Fulminant Hepatic Failure

Either acute or chronic persistent HBV infection may induce fulminant hepatitis. HBV-specific $CD8^+$ T cell response plays a key role in viral clearance and disease pathogenesis. Other factors such as immune complex, complement activation, innate immunity, and ischemia may also contribute to massive hepatic necrosis and hepatic failure.

In the global HBV vaccination era, fulminant acute hepatic failure has become quite rare. Off-therapy and immune suppression-related HBV flares have become the main causes of fulminant hepatic failure. It has developed into an important concern in the treatment of chronic hepatitis B with cessation of NA therapy [20], and in patients receiving immune suppression or chemotherapy [21].

16.12 Chronic Hepatitis, Liver Cirrhosis and Massive Hepatic Necrosis

Persistent viral replication and intermittent inflammation in patients with chronic hepatitis B induces liver fibrosis [22]. Patients with severe flares usually have associated massive hepatic necrosis or so-called bridging hepatic necrosis. They may develop liver cirrhosis within several months (Fig. 16.2) [23]. This rapidly developed liver cirrhosis is quite different from chronic hepatitis C or other chronic liver diseases, which usually require decades to develop into liver cirrhosis. On the other hand, strong immune-related inflammation or long-term NA therapy might induce sustained viral suppression. In such cases, regression of liver fibrosis may be found. An example of this is shown in Fig. 16.2.

Liver cirrhosis is the major risk factor influencing survival in chronic persistent HBV infection [7, 8]. Non-invasive modalities for measurement of liver fibrosis have become an important indicator in screening policy and treatment planning.

Several fibrosis-detecting modalities had been developed. Fibrosis 4 (FIB 4, calculated from age, AST, ALT and platelet count), conventional ultrasound (US), and US-based elastography are the most popular modalities currently in use [24, 25]. They have different cutoff values under different etiologies, degrees of steatosis, and other confounding factors. How to use these modalities in suitable conditions should be considered carefully.

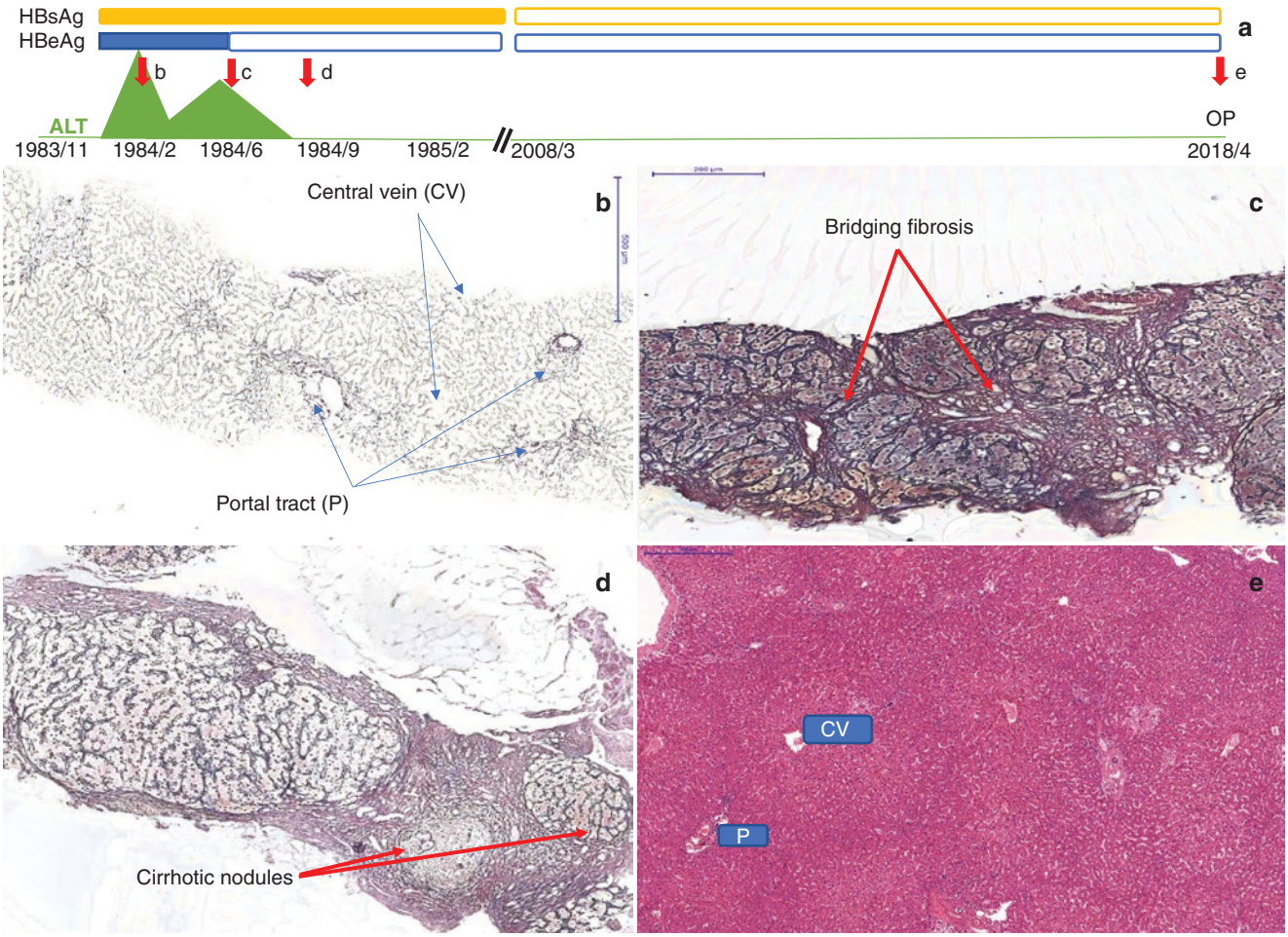

Fig. 16.2 A 22-year-old HBeAg positive female rapidly developed liver cirrhosis that spontaneously resolved 34 years later. (**a**) The clinical course and timing of four liver histology studies are shown; three biopsies were performed during a severe ALT flare-up as part of an Ara-A clinical trial. Viral replication was shut down and became persistent normal ALT after HBeAg seroconversion. She lost to followed-up and return with HBsAg clearance. The last biopsy was collected during a segmentectomy for a progressively enlarged angiomyolipoma which detected during periodic followed-up. (**b**) The initial silver-stained histology section revealed relatively normal reticulum architecture. (**c**) Four months later, severe bridging fibrosis was noted. (**d**) Well-recognized cirrhotic nodules were noted 7 months after the initial biopsy. (**e**) Thirty-four years after HBeAg seroconversion, hematoxylin and eosin staining of the non-tumor portion of the liver biopsy revealed a nearly normal liver with an Ishak fibrosis score of 1–2

16.13 Hepatocellular Carcinoma

Chronic HBV infection is associated with a high risk of liver cancer. Male gender, perinatal infection, old age, long HBV replication phase, HBV integration, liver cirrhosis, personal habits, aflatoxin, drug abuse [26], and environmental factors all contribute to HBV-related hepatocarcinogenesis. A combination of these processes results in an increased HCC risk [1, 4, 9, 10]. The HBV is capable of integration into the human genome, even during the immune tolerance phase. This phenomenon makes it possible for HCC to occur in patients with minimal fibrosis and no evidence of cirrhosis. This is quite different from cases of chronic HCV carriers, or patients with alcoholic or non-alcoholic liver cirrhosis, among which HCC usually develops if they have progressed to cirrhosis.

The molecular mechanisms of liver cancer are complicated and diverse, with different etiologies. Tumor protein p53 (TP53) oncosuppressor and catenin beta 1 (CTNNB1) oncogene are the most frequently mutated genes (31–37%) in HBV-related HCC [27].

Host genetic factors associated with HBV-related hepatocarcinogenesis had been researched intensively, but without reproducible results.

16.14 HBV Vaccination

A nationwide vaccination program has been conducted in Taiwan since 1984. A significant drop in HBsAg prevalence from more than 15% to less than 1% was reported. Maternal viral load greater than 10^8 copies/mL results in a 10% vaccination failure rate in the offspring. A short course of NA therapy starting at the last trimester and ending 1 month after delivery greatly reduced this failure rate [28].

To protect against infection, a course of vaccinations may be needed in adults without previous exposure to HBV. This is especially advisable in those planning travel from low endemic areas to high endemic areas. For those encountering used needles or other materials from HBsAg carriers, a dose of Hepatitis B immunoglobulin should be given as soon as possible followed by HBV vaccination.

16.15 Anti-HBV Therapy

While many drugs have been approved for treatment of chronic hepatitis B, complete virology response (CVR) rates were lower than 30% in most trials [1]. Therefore, according to the guidelines, only patients with elevated ALT level, HBV DNA greater than 10,000 copies/mL, or liver cirrhosis should be treated.

16.16 Pegylated Interferon Alpha (IFN-α) Therapy

For those with HBeAg-positive chronic hepatitis B, the first choice should be pegylated interferon-alpha (IFN-α) therapy for 1 year. This immune modulatory therapy requires weekly intramuscular injections for the duration of the treatment. The HBeAg seroconversion rate 6 months after completing 52 weeks of pegylated IFN-α therapy completion was around 32–36% [29]. IFN-α responders were generally patients with younger age, female gender, ALT elevation, low HBV DNA level, and genotype A or B. There is a relatively higher HBsAg clearance rate (around 5%) associated with IFN-α therapy compared to other anti-HBV therapy [1].

For those with HBeAg-negative chronic hepatitis B, pegylated IFN-α therapy for 1 year is still recommended. The sustained virologic response rate (SVR), as defined by HBV DNA <2000 IU/mL, 6 months after therapy was around 20% [30]. HBsAg seroconversion occurred in 3% of patients.

16.17 Nucleos(t)ide Analogues Therapy

The drug of choice for nucleos(t)ide analogues (NA) therapy in the CHB is entecavir (ETV) or tenofovir disoproxil fumarate (TDF). These drugs are highly effective HBV suppressors with low drug resistance. Drug resistance was a problem for first-generation NA therapy but has ceased to be so in the second generation. ETV (1.2%) and TDF (0%) had low 5-year drug resistance rates in treatment-naïve patients [1].

TDF is currently widely used in chronic hepatitis B, but will be replaced by tenofovir alafenamide fumarate, which has low renal and bone toxicity [31].

NAs are taken orally, have few side effects, and can effectively suppress HBV viral loads and liver inflammation. However, the HBeAg seroconversion rate is dependent on ethnicity and the duration of therapy. Among patients who received 4–5 years of ETV therapy, it was 15–38% in Asians and 55–58% in Europeans [1]. The difference could be related to disparate genetic backgrounds between East Asians and other populations [13]. When only Europeans studies were compared, the TDF (around 35%) had a lower HBeAg seroconversion rate than ETV. Whether a strong HBV suppression may evade immune surveillance remains to be determined through further evaluation.

The duration of NA therapy remains under debate. When NA therapy is stopped, HBV virologic and clinical relapse in the first year occurs with a likelihood of around 50% [32–34]. Some patients may develop a vigorous flare and proceed to hepatic failure [20]. Long-term treatment is required to maintain virologic control in patients with liver cirrhosis. For those patients without liver cirrhosis, stopping NA therapy

after HBV DNA is undetectable for 1 year may be considered, and could promote HBsAg clearance.

16.18 Combination Therapy

Combination therapies of IFN-α and NAs have been studied with different strategies, but so far, none have been conclusively adapted for clinic use. Recently, sequential NA therapy followed by pegylated IFN-α therapy was found to produce a higher HBeAg seroconversion rate (14.9–44% versus 0–6.1%) than monotherapy [29]. In HBeAg negative patients, sequential NA followed by pegylated IFN-α had a higher HBsAg clearance rate (9–11%) than monotherapy (1–3%)

Combination therapy is more effective than monotherapy, but also increases treatment costs. Further studies will be needed to improve understanding of the immune clearance mechanism, develop new therapeutic strategies, and identify new anti-HBV specific agents in order to develop more efficient combination therapies.

16.19 Future Perspectives

Current anti-HBV therapies may suppress rather than eradicate HBV in patients with chronic hepatitis B. Further understanding of the mechanism of immune tolerance, as well as host and HBV interaction, and development of new therapeutic strategies are needed.

16.20 Conclusion

Chronic HBV infection is associated with timing of HBV infection and host genetic background. Such infection is characterized by an initial immune tolerance phase with high HBV replication, followed by an immune clearance phase, and finally a residual phase with low HBV replication. A significant decrease in chronic HBV infection has been achieved through a global HBV vaccination program in neonates. The survival of patients with chronic hepatitis B is also improving with the widespread use of NA therapy. These efforts have decreased fibrogenesis and hepatocarcinogenesis. However, two-thirds of patients relapse 3 years after the end of an NA therapy. There is still an urgent need for new therapeutic strategies, agents, and trials in chronic HBV infection.

Self Study

Questions

1. **Which statement is false?**
   (a) HBV is a highly infectious, small, circular, incomplete double stranded RNA virus.
   (b) Chronic infection is common when infected in the early stages of life.
   (c) The course of chronic HBV infection is a trilogy, starting with immune tolerance, followed by immune clearance, and finally a residual phase.
   (d) Immune tolerance is a survival strategy to avoid cytokine storm. It could be associated with genetic evolution during human migration.
2. **Which statement is true?**
   (a) The severity and duration of liver inflammation determine the development of liver cirrhosis and hepatocellular carcinoma.
   (b) Most chronic HBV carriers may terminate HBV replication, but are unable to achieve delayed HBsAg clearance even several decades later.
   (c) Current HBV-specific therapy may suppress viral replication and clear covalently closed circular DNA in the nucleus.
   (d) Clustering of chronic HBsAg infection in a family is related to HBV transmission, but not to genetic background.

Answers

1. **Which statement is false?**
   (a) HBV is a highly infectious, small, circular, incomplete double stranded DNA virus.
2. **Which statement is true?**
   (a) The severity and duration of liver inflammation determine the development of liver cirrhosis and hepatocellular carcinoma.
   (b) Most chronic HBV carriers may terminate HBV replication, and at age 80, 50% of HBsAg carriers achieve delayed HBsAg clearance.
   (c) Current HBV-specific therapy may suppress viral replication and is unable to clear covalently closed circular DNA in the nucleus.
   (d) Clustering of chronic HBsAg infection in a family is related to HBV transmission and to inherited HLA-DP and -DQ loci.

References

1. Liaw Y-F, Zoulim F, editors. Hepatitis B virus in human diseases. New York: Humana Press; 2016.
2. Wen J, Song C, Jiang D, Jin T, Dai J, Zhu L, et al. Hepatitis B virus genotype, mutations, human leukocyte antigen polymorphisms and their interactions in hepatocellular carcinoma: a multi-centre case-control study. Sci Rep. 2015;5:16489.
3. Lazarevic I. Clinical implications of hepatitis B virus mutations: recent advances. World J Gastroenterol. 2014;20:7653–64.
4. Beasley RP. Rocks along the road to the control of HBV and HCC. Ann Epidemiol. 2009;19(4):231–4.
5. Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. Elife. 2012;1:e00049.
6. Wieland S, Thimme R, Purcell RH, Chisari FV. Genomic analysis of the host response to hepatitis B virus infection. Proc Natl Acad Sci U S A. 2004;101:6669–74.
7. Liaw YF. Impact of therapy on the outcome of chronic hepatitis B. Liver Int. 2013;33(Suppl 1):111–5.
8. Bunchorntavakul C, Reddy KR. Acute liver failure. Clin Liver Dis. 2017;21:769–92.
9. Tai DI, Tsay PK, Chen WT, Chu CM, Liaw YF. Relative roles of HBsAg seroclearance and mortality in the decline of HBsAg prevalence with increasing age. Am J Gastroenterol. 2010;105:1102–9.
10. Tai DI, Lin SM, Sheen IS, Chu CM, Lin DY, Liaw YF. Long-term outcome of HBeAg-negative HBsAg carriers in relation to changes of alanine aminotransferase levels over time. Hepatology. 2009;49:1859–67.
11. Kamatani Y, Wattanapokayakit S, Ochi H, Kawaguchi T, Takahashi A, Hosono N, et al. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat Genet. 2009;41:591–5.
12. Mbarek H, Ochi H, Urabe Y, Kumar V, Kubo M, Hosono N, et al. A genome-wide association study of chronic hepatitis B identified novel risk locus in a Japanese population. Hum Mol Genet. 2011;20:3884–92.
13. Tai DI, Jeng WJ, Lin CY. A global perspective on HBV-related SNPs and evolution during human migration. Hepatol Commun. 2017;1:1005–13.
14. Liaw YF. Clinical utility of HBV surface antigen quantification in HBV e antigen-negative chronic HBV infection. Nat Rev Gastroenterol Hepatol. 2019. https://doi.org/10.1038/s41575-019-0197-8.15.
15. World Health Organization. Guidelines on hepatitis B and C testing, Geneva. 2017. http://www.who.int/hepatitis/publications/guidelines-hepatitis-c-b-testing/en/. Accessed Feb 2017.
16. Li X, Zhao J, Yuan Q, Xia N. Detection of HBV covalently closed circular DNA. Viruses. 2017;9(6). pii: E139.
17. Kennedy PTF, Sandalova E, Jo J, Gill U, Ushiro-Lumb I, Tan AT, et al. Preserved T-cell function in children and young adults with immune-tolerant chronic hepatitis B. Gastroenterology. 2012;143(3):637–45.
18. Mason WS, Gill US, Litwin S, Zhou Y, Peri S, Pop O, et al. HBV DNA integration and clonal hepatocyte expansion in chronic hepatitis B patients considered immune tolerant. Gastroenterology. 2016;151:986–98.
19. Kollmann TR, Levy O, Montgomery RR, Goriely S. Innate immune function by Toll-like receptors: distinct responses in newborns and the elderly. Immunity. 2012;37:771–83.
20. Chang ML, Jeng WJ, Liaw YF. Clinical events after cessation of lamivudine therapy in patients recovered from hepatitis B flare with hepatic decompensation. Clin Gastroenterol Hepatol. 2015;13:979–86.
21. Mandala M, Fagiuoli S, Francisci D, Bruno R, Merelli B, Pasulo L, et al. Hepatitis B in immunosuppressed cancer patients: pathogenesis, incidence and prophylaxis. Crit Rev Oncol Hematol. 2013;87:12e27.
22. Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. Hepatology. 1988;8:493–6.
23. Chen CJ, Tsay PK, Huang SF, Tsui PH, Yu WT, Hsu TH, et al. Effects of hepatic steatosis on non-invasive liver fibrosis measurements between hepatitis B and other etiologies. Appl. Sci. 2019;9:1961.
24. Lim JK, Flamm SL, Singh S, Falck-Ytter YT. Clinical Guidelines Committee of the American Gastroenterological Association. American Gastroenterological Association Institute guideline on the role of elastography in the evaluation of liver fibrosis. Gastroenterology. 2017;152:1536–43.
25. Shiha G, Ibrahim A, Helmy A, Sarin SK, Omata M, Kumar A, et al. Asian-Pacific Association for the Study of the Liver (APASL) consensus guidelines on invasive and non-invasive assessment of hepatic fibrosis: a 2016 update. Hepatol Int. 2017;11:1–30.
26. Ng AWT, Poon SL, Huang MN, Lim JQ, Boot A, Yu W, et al. Aristolochic acids and their derivatives are widely implicated in liver cancers in Taiwan and throughout Asia. Sci Transl Med. 2017;9(412) https://doi.org/10.1126/scitranslmed.aan6446.
27. Ahn SM, Jang SJ, Shim JH, Kim D, Hong SM, Sung CO, et al. Genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. Hepatology. 2014;60:1972–82.
28. Chen HL, Lee CN, Chang CH, Ni YH, Shyu MK, Chen SM, et al. Taiwan Study Group for the Prevention of Mother-to-Infant Transmission of HBV (PreMIT Study); Taiwan Study Group for the Prevention of Mother-to-Infant Transmission of HBV PreMIT Study. Efficacy of maternal tenofovir disoproxil fumarate in interrupting mother-to-infant transmission of hepatitis B virus. Hepatology. 2015;62:375–86.
29. Woo ASJ, Kwok R, Ahmed T. Alpha-interferon treatment in hepatitis B. Ann Transl Med. 2017;5:159.
30. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med. 2004;351:1206–17.
31. Agarwal K, Brunetto M, Seto WK, Lim YS, Fung S, Marcellin P, et al. 96 weeks treatment of tenofovir alafenamide vs. tenofovir disoproxil fumarate for hepatitis B virus infection. J Hepatol. 2018;68:672–81.
32. Jeng WJ, Chen YC, Sheen IS, Lin CL, Hu TH, Chien RN, et al. Clinical relapse after cessation of tenofovir therapy in hepatitis B e antigen-negative patients. Clin Gastroenterol Hepatol. 2016;14:1813–20.
33. Jeng WJ, Sheen IS, Chen YC, Hsu CW, Chien RN, Chu CM, et al. Off-therapy durability of response to entecavir therapy in hepatitis B e antigen-negative chronic hepatitis B patients. Hepatology. 2013;58:1888–96.
34. Chang ML, Liaw YF, Hadziyannis SJ. Systematic review: cessation of long-term nucleos(t)ide analogue therapy in patients with hepatitis B e antigen-negative chronic hepatitis B. Aliment Pharmacol Ther. 2015;42:243–57.