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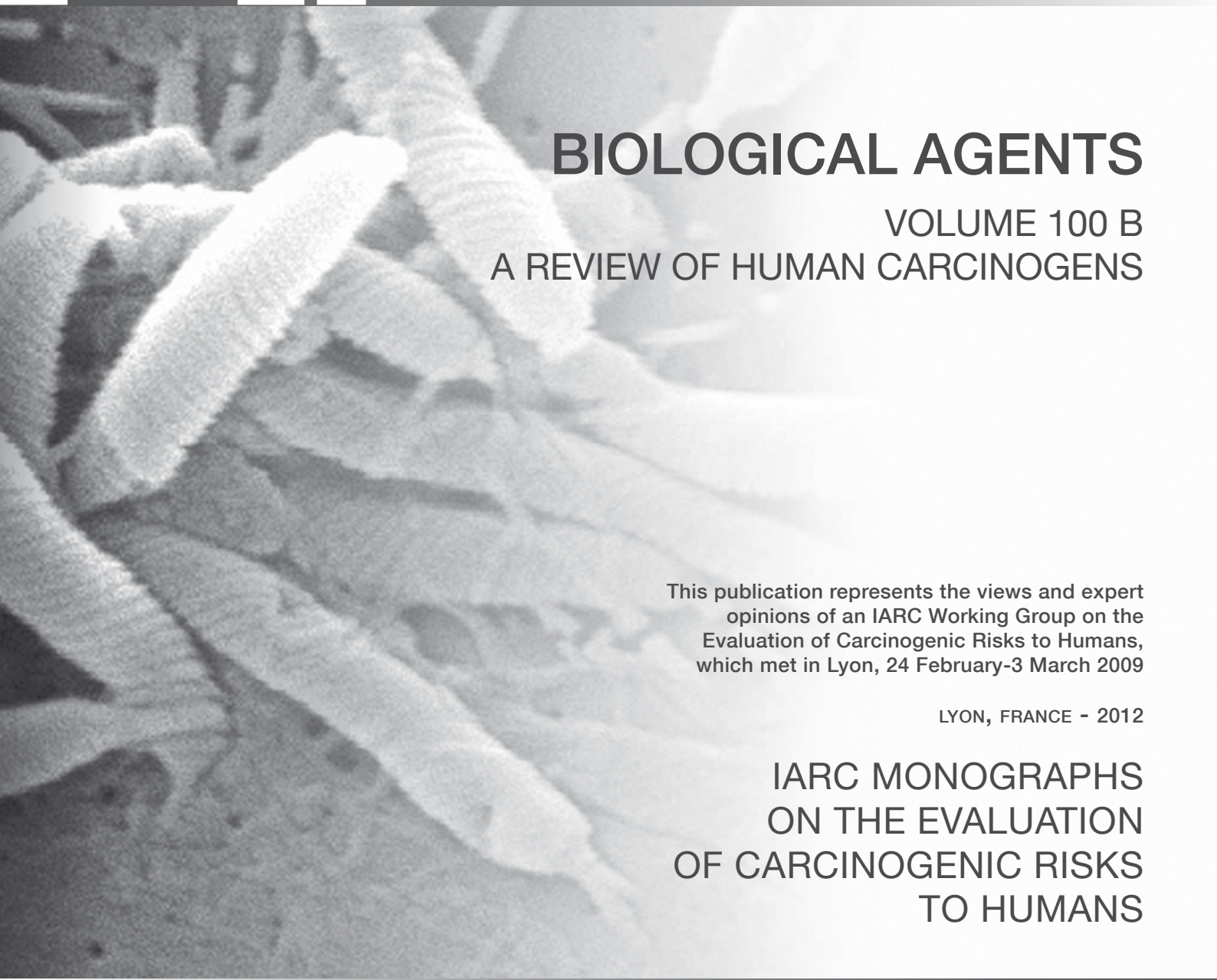

BIOLOGICAL AGENTS

VOLUME 100 B
A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 24 February-3 March 2009

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OF CARCINOGENIC RISKS
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International Agency for Research on Cancer
World Health Organization

HEPATITIS B VIRUS

The hepatitis B virus was considered by a previous IARC Working Group in 1993 (IARC, 1994). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Taxonomy, structure, and biology

1.1.1 Taxonomy

The hepatitis B virus (HBV) is the prototype member of a family of hepatotropic DNA viruses, the *Hepadnaviridae*, that replicate by reverse transcription of an RNA pregenome. HBV infects humans, whereas other hepadnaviruses infect mammals (orthohepadnaviruses) or birds (avihepadnaviruses) (Schaefer, 2007a).

HBV comprises eight genotypes (A to H) with distinct virological characteristics and geographic distributions (Kramvis *et al.*, 2005; Schaefer, 2007a; see Section 1.2.3). Each genotype differs from the others by more than 8% of its nucleotide sequence. Genotypes may influence the disease caused, although further analysis of this association is required. The variability of the HBV genome may be further increased by recombination among genotypes, especially B/C and A/D.

HBV genotypes, with the exception of genotypes E and G, are divisible into subgenotypes. Each subgenotype differs from the others by more than 4% of its nucleotide sequence. The number of subgenotypes per genotype described to date ranges from three to five (Kramvis *et al.*, 2005; Schaefer, 2007a).

1.1.2 Structure of the virion

HBV is an enveloped virus, measuring 42–47 nm in diameter, with an icosahedral nucleocapsid that encloses a partially double-stranded relaxed-circular (rc) DNA genome covalently bound to the viral polymerase. The envelope comprises a small amount of lipid of cellular origin and three hepatitis B surface proteins (HBs): large (LHB), medium, (MHB), and small (SHB), which form disulfide-linked homo- and heterodimers. The serum of infected individuals contains, in addition, two types of subviral particles: small spherical particles with a diameter of approximately 20 nm and filamentous particles also with a diameter of about 20 nm but of variable length. These non-infectious subviral particles lacking genomic DNA greatly outnumber the infectious viral particles, and have a composition similar to that of the viral envelope (Kann, 2002).

The nucleocapsid is formed by multiple copies of core protein. Of the total 183–185 amino acids (depending upon genotype), the *N*-terminal 149–151 amino acids are responsible for self-assembly of the nucleocapsid. Although the steps in its assembly remain to be clarified, the

first step is the formation of homodimers linked by disulfide bridges. The nucleocapsid contains pores that allow the diffusion of nucleotides during the synthesis of the DNA genome. The *C*-terminal amino acids of the core protein play a role in the packaging of the pregenome–polymerase complex within the nucleocapsid (Bruss, 2007).

1.1.3 Structure of the viral genome

HBV has a partially double-stranded but not covalently closed circular (ccc) DNA genome composed of between 3182–3248 nucleotides, depending on the genotype. The genome consists of a complete minus-DNA strand with a short-terminal redundancy, and a shorter plus-DNA strand that leaves a single-stranded gap of variable length in mature nucleocapsids and released viruses (Kann, 2002; Jilbert *et al.*, 2002). Base-pairing of plus- and minus-strands in the cohesive overlap region of the genome maintains the circular configuration. The 5′end of the minus-strand is covalently linked to the *N*-terminal portion of the viral polymerase. At its 5′end, the plus-strand is linked to a capped RNA oligonucleotide that is derived from the 5′end of the RNA pregenome, and serves as the primer for plus-strand-DNA synthesis.

The genome consists of four partially overlapping open reading frames (ORFs) (Kann, 2002) that express surface, precore/core, polymerase, and X proteins. Each ORF overlaps at least one other ORF, with the polymerase ORF overlapping all of the others, and every nucleotide is part of at least one ORF. Translation of preS1, preS2, and S ORFs leads to the expression of the surface proteins, LHB, MHB, and SHB, respectively (Fig. 1.1).

Four promoters (preC/C, preS1, S, and X) and two enhancers (Enh1 and Enh2) overlap the ORFs (Kann, 2002). The promoters initiate the transcription of messenger (m) RNAs of 3.5, 2.4, 2.1 and 0.9 kb that allow, by the use of different start codons, the expression of seven proteins. All are of positive orientation, possess a 5′cap, are polyadenylated at their 3′ends, and serve as mRNAs for viral gene products. Enh1, which stimulates the transcription of all viral RNAs, is located between the S and X ORFs, and Enh2, a less potent enhancer, overlaps the preC/C promoter.

In addition to the enhancers, other regulatory elements have been identified: a glucocorticoid responsive element (GRE) is located between Enh1 and Enh2; a CCAAT element regulates the transcription of the upstream preS1 promoter, and activates the transcription of S mRNA; and a negative regulatory element (NRE) appears to inhibit only the precore/core mRNA (Kann, 2002).

1.1.4 Host range

HBV primarily infects humans, although chimpanzees, Chacma baboons, and tree shrews are also susceptible to infection (Hu *et al.*, 2000; Cao *et al.*, 2003).

1.1.5 Target cells

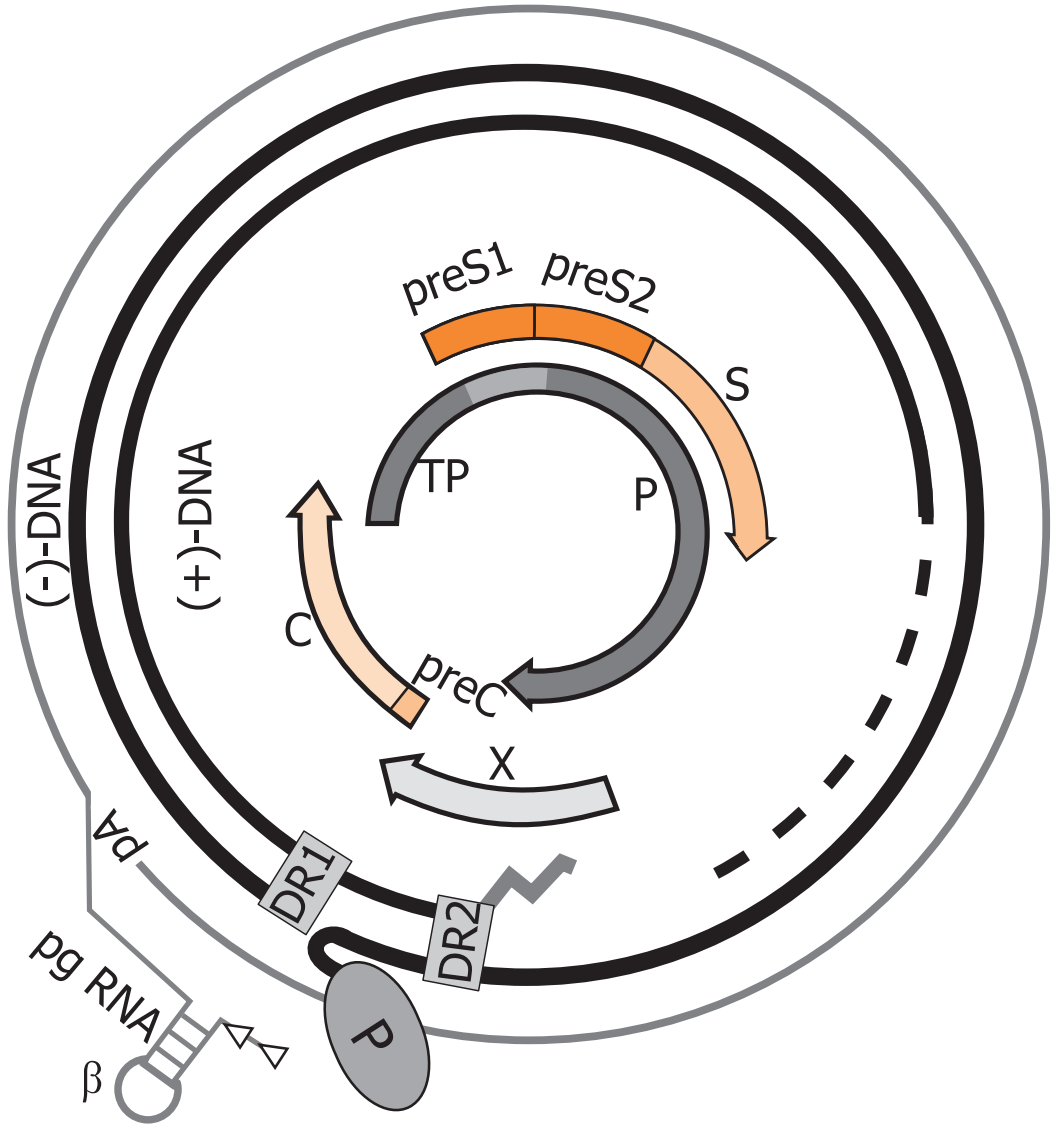
HBV is primarily an hepatotropic virus, and hepatocytes are the only confirmed site of replication for all members of this virus family. Although the virus has been detected in other cells such as bile duct epithelial cells, peripheral blood mononuclear cells and cells in the pancreas and kidneys, the evidence for viral replication in these cells is controversial (Seeger & Mason, 2000).

1.1.6 Function of the gene products

(a) Surface proteins

The HBV surface protein: small (SHB), medium (MHB) and large (LHB), together with cellular lipid material, form the viral envelope (Kann, 2002).

Fig. 1.1 Transcriptional and translational map of HBV



The partially double-stranded, circular rc-DNA is indicated by thick black lines, with the polymerase (P) covalently linked to the 5′end of the (-)-DNA, and the RNA primer (zigzag line) at the 5′end of (+)-DNA. The dashed part symbolizes the heterogeneous lengths of the (+)-strands. DR1 and DR2 are the direct repeats. The outer circle symbolizes the terminally redundant pgRNA with ε close to the 5′end, and the poly-A tail at the 3′end. The precore mRNA is nearly identical, except it starts slightly upstream. The relative positions of the open reading frames for core (C), P, preS/S, and X are shown inside. TP, Terminal protein domain of P; pgRNA, pregenomic RNA
From Beck J, Nassal M, Hepatitis B virus replication, World J Gastroenterology, 2007; 13(1):48-64

SHB antigen which represents 85% of hepatitis B surface antigen (HBsAg), is highly immunogenic and provokes the host's immune response to HBV. Excess surface protein circulating in subviral particles is thought to dilute the host's immunological response to the virus.

LHB, in contrast to MHB, is essential for infection and viral morphogenesis. It represents 10–30% of the HBsAg of virions and filaments. LHB plays a role in viral entry into hepatocytes, although SHB may also be needed in this process (Kann, 2002).

(b) Core protein and 'e' antigen

Core protein (C) is the major structural component of the nucleocapsid. The preC/C ORF is transcribed into a precore/core fusion protein. During entry into the endoplasmic reticulum, 19 amino acids are cleaved from the *N*-terminal end of the precore protein by a signal peptidase. When transported into the Golgi compartment, additional amino acids are removed from the *C*-terminal end by intra-Golgi proteases to form HBe antigen. This antigen is secreted into the serum. The biological function of HBe remains unsolved (Kann, 2002).

(c) Polymerase protein

Polymerase (P) has four domains: a terminal domain, which serves as a protein primer for reverse transcription of pregenomic viral RNA; a spacer region without apparent function; the polymerase domain, which has reverse transcription activity; and the RNase H domain, which is responsible for the degradation of the RNA template during reverse transcription (Kann, 2002).

(d) X protein

The X protein (HBx) has been shown to be a promiscuous regulator of transcription that is essential for viral replication. Although not binding itself to DNA, it regulates transcription from HBV enhancers/promoters, and from the promoters of cellular genes, including oncogenes, cytokines, growth factors, and several genes involved in cell-cycle control and progression, DNA repair, apoptotic cell death, and cellular adhesion. HBx also forms complexes with several signal transduction proteins and regulators of cell growth and survival (Murakami, 1999; Feitelson *et al.*, 2005; Benhenda *et al.*, 2009). It is suspected to play a central role in HBV regulation and pathogenesis (see Section 4).

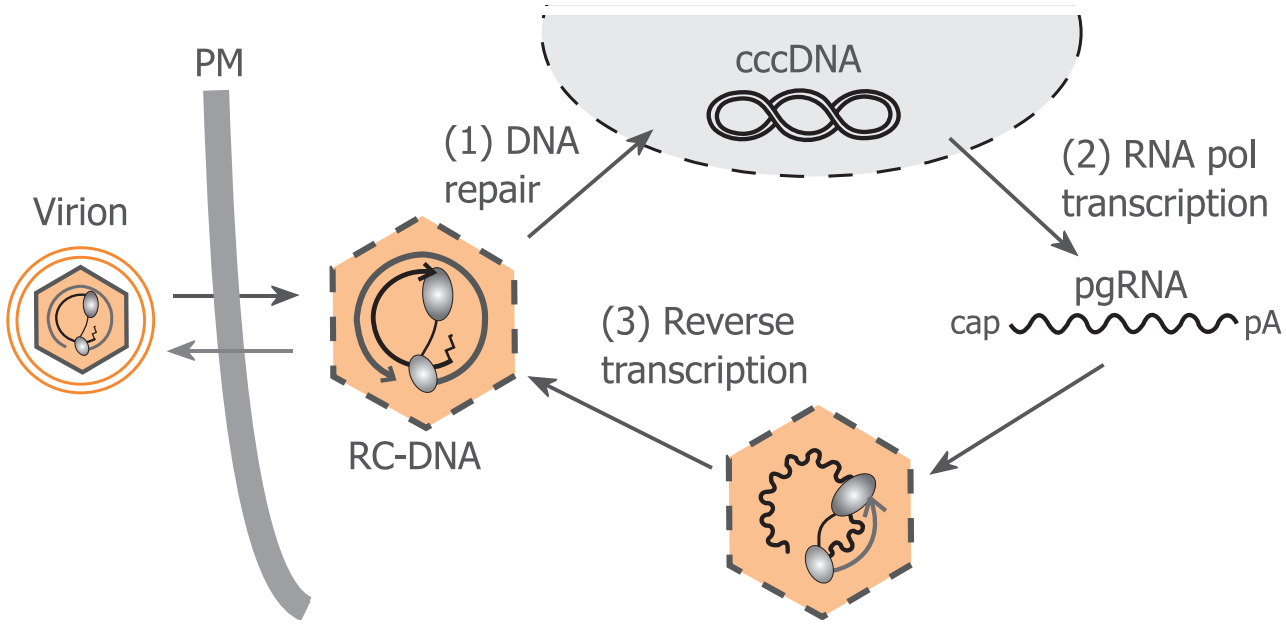
1.1.7 Viral life cycle

During both acute and persistent infection, high levels of infectious HBV particles (virions) circulate in the bloodstream, together with an excess of empty particles.

Hepatocytes, the major targets of the virus, are separated from the bloodstream by endothelial and Kupffer cells that line the sinusoids of the liver. Liver sinusoidal endothelial cells have long cytoplasmic components that contain fenestrations with a diameter of 50–100 nm. Virions are thought to pass through these fenestrations from the sinusoids of the liver to the space of Disse, which is immediately adjacent to the surface of the hepatocytes. Infectious virions bind by means of the PreS1 domain of LHBs (and perhaps by the envelope lipid) to specific, as yet unidentified, receptors on the hepatocyte surface (Seeger & Mason, 2000; Jilbert *et al.*, 2002; Beck & Nassal, 2007; Kann *et al.*, 2007).

HBV then proceeds following a characteristic replication strategy shared by all the members of the hepadnaviridae family (Fig. 1.2; Seeger & Mason, 2000; Jilbert *et al.*, 2002; Beck & Nassal, 2007; Kann *et al.*, 2007). The nucleocapsid is released into the cytoplasm and translocated by microtubules to the microtubule-organizing centre (MTOC) near the nucleus. How the nucleocapsid gets from the MTOC to the nucleus is not known at the time of writing. Access to the nucleus is gained through nuclear pores (Kann

Fig. 1.2 Replication cycle of Hepanaviral genome



Enveloped virions infect the cell, releasing rc-DNA containing nucleocapsids into the cytoplasm. rc-DNA is transported to the nucleus, and repaired to form cccDNA (1). Transcription of cccDNA by RNA polymerase II (2) produces, among other transcripts (not shown), pgRNA. pgRNA is encapsidated, together with P protein, and reverse transcribed inside the nucleocapsid (3). (+)-DNA synthesis from the (-)-DNA template generates new rc-DNA. New cycles lead to intracellular cccDNA amplification; alternatively, the rc-DNA containing nucleocapsids are enveloped and released as virions. PM, plasma membrane; pgRNA, pregenomic RNA; cccDNA, covalently closed circular DNA; rc-DNA, relaxed-circular DNA; P, viral polymerase.
From Beck J, Nassal M, Hepatitis B virus replication, World J Gastroenterology, 2007; 13(1):48-64

et al., 2007), and may be mediated by polymerase or heat shock proteins. The exact stage and mechanism by which the viral genome is released from the nucleocapsid is not currently known. In the nucleus, rc-DNA is converted into cccDNA, the key template in HBV replication. The steps in achieving this conversion are uncertain, but they include completion of the positive DNA strand by polymerase; removal of the covalently linked polymerase, the 5′capped oligonucleotide primer (and the terminal redundancy), and ligation of the 5′ and 3′ends of the positive and negative DNA strands.

Several genomic and subgenomic RNAs are transcribed by cellular RNA polymerase II using cccDNA as the transcriptional template. Of these, the polyadenylated pregenomic RNA, with a length corresponding to the entire genome length plus a terminal redundancy of 120 nucleotides, is selectively packaged into nucleocapsids. It is then reverse-transcribed by the co-packaged polymerase into new rc-DNA genomes. Following encapsidation of the pregenomic RNA-polymerase complex, polymerase initiates negative-strand DNA synthesis by reverse transcription (Jilbert *et al.*, 2002).

The synthesis of polymerase and core proteins is accomplished by two translation events (Jilbert *et al.*, 2002). The mechanism that allows translation from the downstream polymerase protein start codon is not yet known, but may include a direct internal ribosomal entry-site-like binding of the ribosomal subunits at, or near to, the polymerase start codon, “leaky’ scanning of the ribosomes that allow passage to the downstream start codon, or the presence of a ‘minicistron’ upstream of the polymerase ORF that is translated (Jilbert *et al.*, 2002).

1.2 Epidemiology of infection

HBV is one of the most common infectious viruses worldwide. It is estimated that more than two billion people are infected. Approximately 360 million of these are chronically infected (Lee, 1997; Chen *et al.*, 2007a; Dienstag, 2008). Approximately one million people die each year from HBV-related chronic liver disease, including liver cirrhosis and hepatocellular carcinoma (HCC) (Mahoney, 1999). HCC is one of the most common cancers in the world, and chronic HBV infection is responsible for 50–90% of HCC in high-risk areas (Chen *et al.*, 1997).

1.2.1 Prevalence, geographic distribution

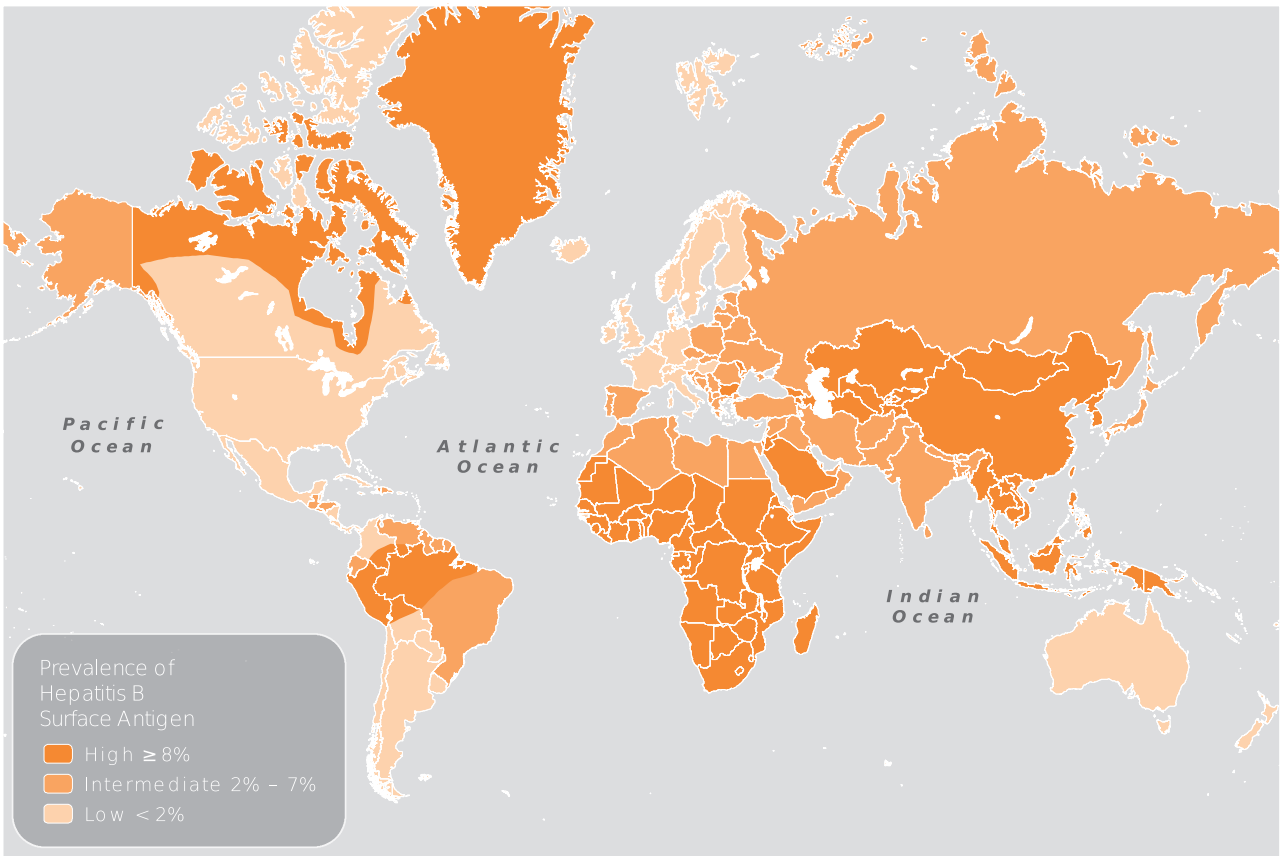
There is a wide variation of HBV infection in the world as shown in Fig. 1.3 (Custer *et al.*, 2004; CDC, 2012). Approximately 45% of the world population lives in areas where chronic HBV infection is highly endemic (> 8% of the population are HBsAg-positive); 43% live in areas where endemicity is intermediate (2–7% HBsAg-positive); and 12% live in areas where endemicity is low (< 2% HBsAg-positive). The prevalence of chronic HBV infection is lowest in North America, Northern and Western Europe, Australia and New Zealand; intermediate in Japan, the Middle East, Eastern and Southern Europe and parts of South America; and highest in sub-Saharan Africa, the Amazon Basin, the People's Republic of China, the Republic of Korea, Taiwan (China), and several other countries in South-east Asia (Chen *et al.*, 2000; Custer *et al.*, 2004).

The worldwide variation in the endemicity of HBV infection is influenced primarily by the predominant age at which infection occurs and the modes of transmission by which it occurs. In areas of high endemicity, the lifetime risk of HBV infection is more than 60%, and most infections are acquired from perinatal and child-to-child transmission, when the risk of developing chronic infection is greatest. In these areas, acute hepatitis B is uncommon because most perinatal and early childhood infections are asymptomatic. However, rates of liver cancer and cirrhosis in adults are very high. Chronic carriage is thought to result from vertical transmission in China, Taiwan (China), and the Republic of Korea (Chen *et al.*, 2000). Of note, HBV infection in newborns is less common in Africa. A lower prevalence of HBeAg positivity has been observed in mothers from sub-Saharan Africa compared with mothers in Asia. Child-to-child horizontal transmission accounts for high hepatitis B infection in this region of Africa.

In areas where endemicity is intermediate, mixed patterns of transmission exist, including infant, early childhood, and adult transmission. In low endemicity areas, most HBV infections occur in adolescents and young adults with relatively well defined high-risk groups, including injection drug users, homosexual males, health care workers, and patients who require regular blood transfusion or haemodialysis. In countries where adult horizontal transmission patterns are the principal transmission routes, the incidence of HBV infection is highest in adults (Custer *et al.*, 2004).

HBV is a prototype member of the hepadnavirus family. Currently, eight genotypes of HBV (A through H) have been identified on the basis of greater than 8% nucleotide divergence over the whole genome (Devesa *et al.*, 2004). Genotype A is prevalent in Europe, Africa, and North America. Genotype B is prevalent in Taiwan (China), China, Thailand, South-east Asia, and genotype C is prevalent in China, Japan, the Republic of Korea, and South-east Asia. Genotype D is predominant in India, Mediterranean areas, and the Middle East region. Genotype E is limited to West Africa. Genotypes F and G are mostly found in Central and South America. Genotype H has been observed in Mexico and Central America (see Table 1.1).

Fig. 1.3 Prevalence of chronic infection with hepatitis B virus, 2006



Source: CDC (2012). Available at: http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/hepatitis-b.htm

Compared with patients infected with the HBV genotype B, those infected with genotype C have a significantly lower rate of spontaneous HBeAg seroconversion (Furusyo *et al.*, 2002; Kao *et al.*, 2004), a higher histological activity index of necroinflammation or fibrosis score (Lindh *et al.*, 1999; Chan *et al.*, 2002; Kobayashi *et al.*, 2002; Lee *et al.*, 2003a), and a higher risk of developing acute exacerbations (Chu *et al.*, 2002; Kao *et al.*, 2004), reactivation of HBV (Chu & Liaw, 2005, 2007), end-stage liver disease (Kao *et al.*, 2000; Chan *et al.*, 2003; Chu & Liaw, 2005), and HCC (Yu *et al.*, 2005; Yang *et al.*, 2008).

1.2.2 Transmission and risk factors for infection

HBV is highly contagious and is transmitted by percutaneous and permucosal exposure to infected blood and other body fluids (i.e. semen and vaginal fluid). The highest concentrations of the virus occur in blood and wound secretions (WHO, 2001). Moderate concentrations of HBV are found in semen and vaginal fluid, and lower concentrations occur in saliva. HBV is not spread by air, food, or water. Common modes of transmission include mother-to-infant, child-to-child, unsafe injection practices and blood transfusions, and sexual contact. HBV may be

Table 1.1 Global distribution of HBV genotypes

Region/Country	Authors, Year	Study subjects	HBV genotypes
Asia/Taiwan, China	Kao *et al.* (2000)	100 Asymptomatic carriers & 170 patients with histologically verified chronic liver disease and HCC	A:4%, **B:53%**, C:32%, D:5%, F:5%, unclassified:1%
Asia/Taiwan, China	Liu *et al.* (2002)	122 Patients with chronic HBV	A:< 1%, **B:57%**, C;39%, F:4%
Asia/Taiwan, China	Lee *et al.* (2003a)	265 Patients with chronic HBV infection	A:1%, **B:60%**, C:34%, D:2.5%, unclassified:2.5%
Asia/Taiwan, China	Yu *et al.* (2005)	154 HCC cases and 316 matched controls	Among control group: **B:82%**, C:15%
Asia/Taiwan, China	Yang *et al.* (2008)	2762 HBsAg carriers	**B:64%**, C:32%, B+C:4%
Asia/Japan	Usuda *et al.* (1999)	514 HBsAg-positive blood donors	A:5%, B:38%, **C:55%**, D:0.4%, F:0.6%
Asia/Japan	Orito *et al.* (2001)	720 Patients with chronic HBV infection	A:2%, B:12%, **C:85%**, D:0.4%, mixed type:1%
Asia/Japan	Kobayashi *et al.* (2002)	1077 Patients with chronic hepatitis B	A:2%, B:9%, **C:88%**, D:0.2%, F:0.2%, unclassified:0.6%
Asia/China	Ding *et al.* (2001)	97 Asymptomatic HBV carriers, 46 chronic hepatitis, 37 liver cirrhosis and 44 HCC patients in Shanghai	A:1%, B:17%, **C:81%**
Asia/China	Zeng *et al.* (2005)	1096 Chronic HBV carriers from nine provinces in China	B: 41%, **C:53%**, A and D: rare
Asia/China	Zhu *et al.* (2008)	101 HBeAg(-) patients in Hong Kong Special Administrative Region, Shanghai, Beijing	B:36%, **C:64%**
Asia/Hong Kong Special Administrative Region	Yuen *et al.* (2004)	776 Asymptomatic HBsAg carriers	B: 33%, **C:63%**, mixed type:4%
Asia/Republic of Korea	Kim & Song (2003)	65 Patients with chronic HBV infection	**C:100%**
Asia/Republic of Korea	Song *et al.* (2005)	200 Patients with chronic HBV infection	**C:100%**
Asia/Republic of Korea	Kim *et al.* (2007)	209 Patients with chronic HBV infection (107 in Seoul and 102 in Jeju)	**C2 (100%)**
Asia/Thailand	Sugauchi *et al.* (2002)	107 Hepatitis B carriers	B:25%, **C:72%**, D:3%
Asia/Thailand	Tangkijvanich *et al.* (2005)	93 Asymptomatic carriers, 103 chronic hepatitis patients, 60 cirrhosis patients, 76 HCC patients	B:21%, **C:73%**
Asia/Thailand	Jutavijittum *et al.* (2006)	216 HBsAg-positive voluntary blood donors	A:0.5%, B:7%, **C:89%**; B+C:2%
Asia/Thailand	Suwannakarn *et al.* (2008)	147 Asymptomatic HBsAg and HBV DNA carriers	A:1%, B:12%, **C:87%**
Asia/Philippines	Sakamoto *et al.* (2006)	32 Chronic hepatitis patients, 37 cirrhosis patients, 31 HCC patients	**A:51%**, B:22%, C:27%
Asia/Viet Nam	Toan *et al.* (2006)	375 HBV-infected (289 symptomatic, 29 on haemodialysis, 86 asymptomatic)	A:18%, B:10%, **C:25%**, D:20%, E:4%, F:2%, G:5%
Asia/India	Thakur *et al.* (2002)	130 Patients with chronic HBV infection	A:46%, **D:48%**, A+D:6%
Asia/India	Gandhe *et al.* (2003)	19 Asymptomatic carriers 30 chronic hepatitis B patients 8 acute hepatitis B patients 5 fulminant hepatitis B patients	**D:92%**

Table 1.1 (continued)

Region/Country	Authors, Year	Study subjects	HBV genotypes
Asia/India	Vivekanandan *et al.* (2004)	122 Chronic hepatitis B patients and 67 blood donors	A:18%, C:12%, **D:57%** among chronic hepatitis B patients A:12%, C:0%, **D:76%** among blood donors
Asia/India	Thippavazzula *et al.* (2006)	85 Chronic hepatitis B patients	A:15%, B:2%, **D:82%**
Mediterranean/Turkey	Yalcin *et al.* (2004)	32 Chronic hepatitis B patients and 12 HBsAg carriers	**D:100%**
Mediterranean/Turkey	Bozdayi *et al.* (2004)	41 Chronic hepatitis B patients	**D:100%**
Mediterranean/Turkey	Sunbul & Leblebicioglu (2005)	88 Chronic hepatitis B patients	**D:89%**
Africa/Nigeria	Odemuyiwa *et al.* (2001)	20 New isolates of HBV	**E:100%**
Africa/West Africa	Mulders *et al.* (2004)	105 Strains from 12 locations in West Africa	**E:91%**
Africa	Kramvis & Kew (2007)	Literature review	**A:** predominantly in southern, eastern and central Africa **D:** predominantly in northern Africa **E:** predominantly in western Africa
America/USA	Chu *et al.* (2003)	694 Chronic hepatitis B patients	**A:35%**, B:22%, C:31%, D:10%, E:0.4%, F:0.6%, G: 1%
America/Central	Arauz-Ruiz *et al.* (1997)	90 Strains from 5 different countries in Central America (Guatemala, El Salvador, Honduras, Nicaragua and Costa Rica)	A:14%, C:1%, D:6%, **F:79%**
America/Mexican	Sánchez *et al.* (2007)	42 Chronic or acute hepatitis B patients	A:5%, D:21%, **H:74%**
Europe	Schaefer (2007b)	Literature review	**A:** prevalent in northern Europe **D:** prevalent in Mediterranean countries and eastern Europe
Australian	Sugauchi *et al.* (2001)	5 Australian Aborigines	C:40%, **D:60%**
Australian	Alestig *et al.* (2001)	5 Australian Aborigines	**C:100%**
World	Lindh *et al.* (1997)	187 HBeAg-positive chronic carriers	northern Europeans: **A:60%**, D:31% southern Europeans and Middle Easterners: **D:96%** Africans: **A:53%**, D:27%, E:20% East Asians: A:14%, **B:43%, C:43%**
World	Westland *et al.* (2003)	694 Chronic hepatitis B patients in clinical trial centres	Asian/Oceanic centres: B:2%, **C:46%** North American centres: A:34%, **C:40%** Mediterranean centres: A: 14%, **D:83%** European centres: **A:40%,** D:35%

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HBeAg, hepatitis B 'e' antigen; HbsAg, hepatitis B surface antigen
Compiled by the Working Group

detected in serum 30–60 days following infection, and may persist for widely variable periods of time.

Perinatal transmission from HBsAg-positive mothers to their newborn infants (vertical) or transmission from one child to another (horizontal) is a major source of HBV infections in many countries where chronic HBV infection is highly endemic (WHO, 2001). Perinatal transmission usually happens at the time of birth; in-utero transmission is relatively rare, accounting for less than 2% of perinatal infections in most studies. There is no evidence that HBV can be spread by breastfeeding (Beasley *et al.*, 1975). The risk of perinatal transmission depends on the HBeAg serostatus of the mother. The risk of HBV infection approximately ranges from 70–90% for HBeAg-positive mothers to 5–20% for HBeAg-negative mothers (Okada *et al.*, 1976; Beasley *et al.*, 1977). The spread of HBV from child to child usually happens in household settings but may also occur in child daycare centres and schools (WHO, 2001). The most probable pathways of child-to-child spread involve contact of skin sores, small breaks in the skin, or mucous membranes with blood or skin sore secretions (Margolis *et al.*, 1997). HBV may also spread because of contact with saliva through bites or other breaks in the skin, and as a consequence of the premastication of food (MacQuarrie *et al.*, 1974; Scott *et al.*, 1980; Beasley & Hwang, 1983; Williams *et al.*, 1997). The virus may spread from inanimate objects such as shared towels or toothbrushes, because it can survive for at least 7 days outside the body, and can be found in high titres on objects, even in the absence of visible blood (Petersen *et al.*, 1976; Bond *et al.*, 1981; Martinson *et al.*, 1998). Among Gambian children aged 6 months to 5 years, a significant association was observed between HBV infection and the presence of bedbugs in each child's bed (Vall Mayans *et al.*, 1990). But controlling bedbugs by insecticide spraying of the child's dwelling did not have any effect on HBV infection (Vall Mayans *et al.*, 1994).

Unsafe injection practices such as the re-use of a syringe or needle from patient to patient without sterilization are a common source of transmission of HBV in many developing countries (Kane *et al.*, 1999; Simonsen *et al.*, 1999). In addition, unsatisfactory infection control practices, including the re-use of contaminated equipment for medical, cosmetic or dental procedures, failure to use appropriate disinfection and sterilization practices for equipment and environmental surfaces, and improper use of multidose medication vials, can also result in the transmission of HBV. Blood transfusion is also a common source of HBV transmission in countries where the blood supply is not screened for HBsAg. In addition, the injection of illicit drugs using shared needles is a common mode of HBV transmission in many developed countries.

HBV is efficiently transmitted by sexual contact, which accounts for a high proportion of new infections among adolescents and adults in countries with low and intermediate endemicity of chronic HBV infection (Alter & Margolis, 1990). Risk factors for sexual transmission include multiple sexual partners, prostitution, and lack of protection in sexual activity (e.g. the use of condoms). In countries where HBV infection is highly endemic, sexual transmission does not account for a high percentage of cases because most persons have been infected since childhood.

1.2.3 Persistence, latency, and natural history of infection

Persons infected with HBV have both short-term and long-term outcomes. On becoming infected, a person can have either a symptomatic disease (i.e. acute hepatitis B), or an asymptomatic infection with no signs or symptoms of disease. In persons with acute hepatitis B, the incubation period after becoming infected is usually

3–4 months, with a range of 6 weeks to 6 months. Symptoms and signs of disease usually last for several weeks. About 1–2% of persons with acute hepatitis B die from fulminant hepatitis. Both symptomatically and asymptomatically infected persons may either recover from the infection and develop lifelong immunity, or develop a chronic infection that usually lasts throughout life. Persons affected with chronic infection often do not become sick from their infection for decades after becoming infected. However, about 25% of those who become chronically infected during childhood and 15% of those who acquire chronic infection at older ages develop either HCC or cirrhosis.

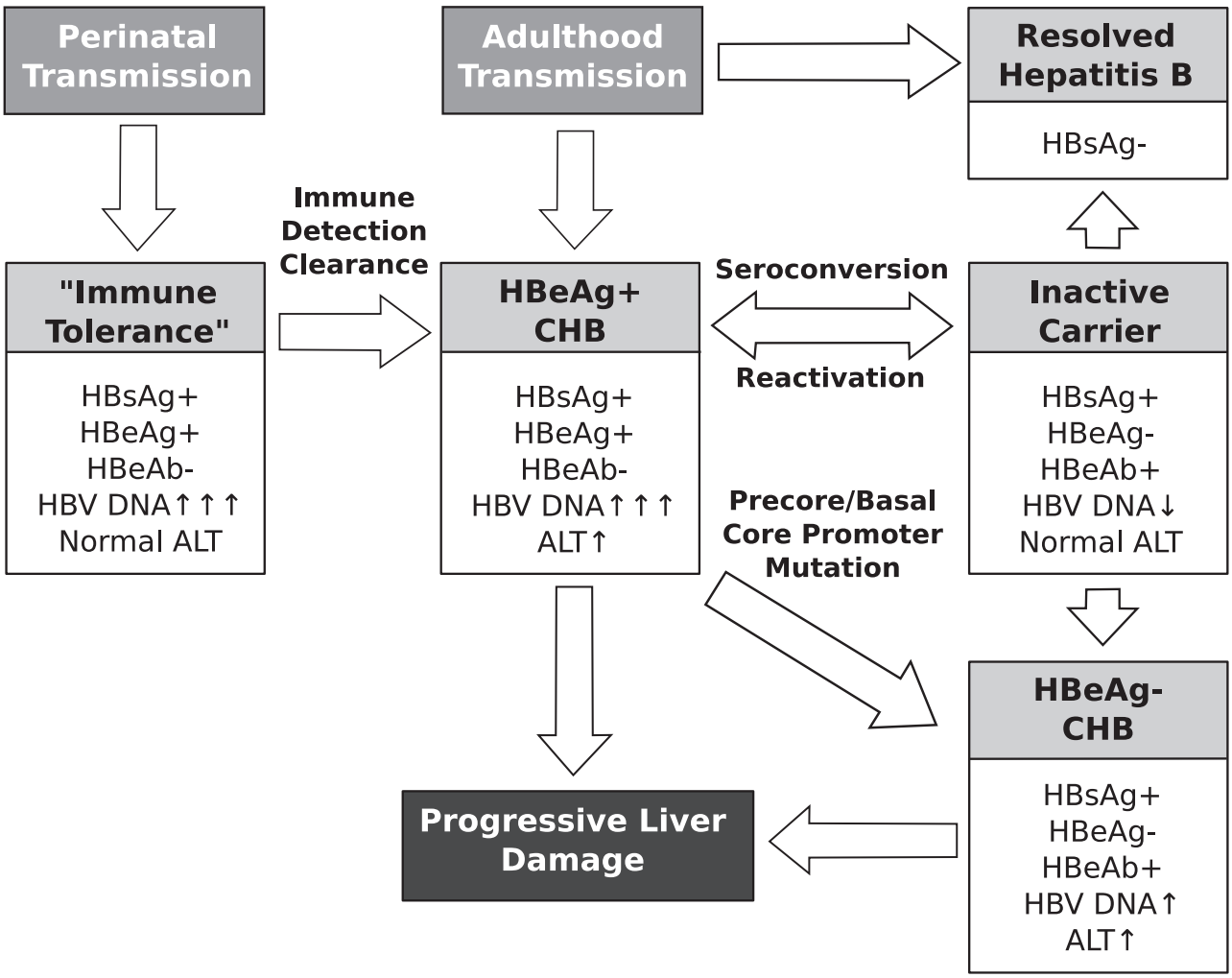
The age at which a person becomes infected with HBV is the main factor determining the risk of developing chronic infection. Among children who are under 5 years of age when they become infected, fewer than 10% are symptomatic. However, 80–90% of those infected infants and 30–50% of children infected between 1–4 years of age develop a chronic infection. In contrast, 30–50% of adults are symptomatic when first infected but only 2–5% of adults develop a chronic infection. Most of the disease burden associated with HBV infection is in persons who develop the chronic condition.

Thus, the natural course of chronic HBV infection is highly variable at an individual level but also varies with age of infection. The classical description of the natural history of chronic HBV infection is shown in Fig. 1.4 (Chen *et al.*, 2007a; Dienstag, 2008). Early life/perinatal infection is characterized by a period of 'immune tolerance' where the host co-exists with the virus without apparent injury to the host. This period of immune tolerance is characterized by detectable circulating HBsAg, HBeAg, the absence of anti-HBe antibody, high levels of circulating HBV DNA and normal serum alanine aminotransferase (ALT). This immune tolerance may last for years generally without evidence of liver injury.

Following the immune tolerance phase, infected patients progress through a phase of immune detection/clearance where the host immune system tries to clear infected hepatocytes resulting in hepatic inflammation, elevation of serum ALT, and reduction of the circulating HBV DNA level. The immune clearance phase is highly variable in duration and frequency but a prolonged phase or recurrent episodes of acute liver inflammation may result in repeated cycles of injury and regeneration, resulting in necroinflammation/fibrosis and an increased risk of progression to cirrhosis and HCC. In some cases, conversion to anti-HBe-seropositive status follows the immune clearance phase. The progression of chronic hepatitis B to a state of detectable liver injury represents the start of the disease state, which is characterized by the presence of HBsAg and HBeAg in serum (HBeAg-positive chronic hepatitis B), moderate-to-high levels of circulating HBV DNA, elevation of serum ALT, and the absence of anti-HBe antibody. In some cases, where seroconversion to anti-HBe-seropositive status is associated with ongoing viral replication, there is detectable anti-HBe antibody (HBeAg-negative chronic hepatitis B). In these HBeAg-negative–anti-HBe-positive hepatitis B cases, the HBV DNA level in serum is usually lower than in HBeAg-positive chronic hepatitis B (Chen *et al.*, 2007a).

Finally, a proportion of infected persons will be able to inactivate the infection and go into the 'non-replicative phase' of chronic HBV infection or what is sometimes referred to as the 'inactive carrier state'. This phase is characterized by the continued presence of HBsAg in serum, the absence of HbeAg, and the presence of anti-HBe antibody, low levels of serum HBV DNA, and normal serum ALT. The patients in the inactive carrier state do not usually progress to liver injury. This may in part be dependent on the events that occurred during the immune clearance phase and the presence or absence of pre-existing liver fibrosis. Most of adult infections

Fig. 1.4 Natural history of chronic hepatitis B virus infection



Courtesy of WR Kim MD, Mayo Clinic, Rochester Minnesota

CHB: Chronic hepatitis B, HBsAg: hepatitis B surface antigen; HBeAg: hepatitis Be antigen; HBeAb: anti-HBe antigen; ALT: alanine aminotransferase; HBV DNA: hepatitis B viral DNA

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resolve spontaneously, and the few patients (approximately 5%) who do not clear the infection progress directly to the chronic infection phase, and do not experience an immune tolerance phase (Chen *et al.*, 2007a).

A specific group of patients who are seronegative on HBsAg but seropositive on HBV DNA has been identified, and their infection defined as occult hepatitis B (Hu, 2002; Torbenson & Thomas, 2002; Chen, 2005). Although occult hepatitis B has long been documented (Hoofnagle *et al.*, 1978), it was difficult to investigate it before the availability of HBV polymerase chain reaction (PCR). The molecular and immunological mechanisms underlying occult hepatitis B still remain incompletely elucidated. Several

hypotheses have been proposed for the occurrence of occult HBV infection. They include the mutation of HBV surface, core and X genes, the integration of HBV DNA into host genomes, the HBV infection of peripheral blood mononuclear cells, the formation of the circulating immune complex containing HBV, the altered host's immune response to HBV, and the superinfection and interference of HBV by other viruses. Persons with occult HBV infection may transmit HBV through transfusion, haemodialysis, and organ transplantation. Occult HBV infection may contribute to the acute exacerbation of co-existing chronic hepatitis B and even fulminant hepatitis, and to the development of HCC. It also affects disease progression and treatment response of chronic hepatitis C (Hu, 2002; Torbenson & Thomas, 2002).

There is a wide variation in the prevalence of occult hepatitis B among various patient groups, blood and organ donors, and healthy controls. The prevalence of seropositivity of HBV DNA in HBsAg-seronegative subjects is in the range of 0–10% among those without liver disease, 11–19% in patients affected with chronic hepatitis, and 12–61% in HCC patients (Bréchot *et al.*, 2001; Chen, 2005).

1.2.4 Vaccination and viral treatment

Both vaccine and antiviral treatments are available for the control of HBV infection. The HBV vaccination programme has reduced the perinatal and horizontal transmission of HBVs and the prevalence of HBsAg in many countries including Taiwan (China) (Tsen *et al.*, 1991; Hsu *et al.*, 1999; Ni *et al.*, 2001, 2007; Lin *et al.*, 2002, 2003; Chien *et al.*, 2006; Lu *et al.*, 2006; Su *et al.*, 2007, 2008), Saudi Arabia (Al-Faleh *et al.*, 1999; Madani, 2007), southern Italy (Da Villa *et al.*, 1998), and Senegal and The Gambia (Vildósola, 2000). HBV vaccination has been shown to result in a dramatic decrease in the number of HBV infections among health care workers (Mahoney *et al.*, 1997). It has been well documented that national HBV vaccination programmes have reduced the mortality in childhood fulminant hepatitis (Kao *et al.*, 2001; Chien *et al.*, 2006), and HCC incidence (Chang *et al.*, 1997, 2000, 2005; Chien *et al.*, 2006).

Alpha-interferon and nucleotide/nucleoside analogues have been used to treat patients affected by chronic hepatitis B. Randomized controlled trials have shown the efficacy of antiviral treatment to improve the histological grade and to reduce the risk of liver cirrhosis and HCC (Dienstag, 2008).

2. Cancer in Humans

This section reviews the epidemiological data published since the previous *IARC Monograph* (IARC, 1994). The current review only focuses on cohort and case–control studies with the exception of those descriptive studies which may reflect the effect of hepatitis B vaccination. Many of these studies have not focused primarily on hepatitis B, but on other factors that potentially interact with hepatitis B in causing HCC.

2.1 Hepatocellular carcinoma

The previous *IARC Monographs* concluded that chronic HBV infection was associated with an increased risk of HCC in humans. The conclusion was based primarily on 15 cohort studies and several dozens of case–control studies conducted mostly in Asia and Africa, and some in Europe and North America. In most studies, chronic infection with HBV was determined by the presence of HBsAg positivity in serum. In all cohort studies reviewed, the relative risks ranged from 5.3–148. The majority of the case–control studies examined also showed a strong association. The odds ratios varied between 5–30, although the quality of some case–control studies was variable. This association did not appear to be confounded

by the presence of aflatoxin, infection with HCV, cigarette smoking or alcohol drinking. The evaluation of an association between the risk of HCC and the presence of other serological markers for HBV infection, such as antibody to hepatitis B core antigen (anti-HBc), and antibody to hepatitis B surface antigen (anti-HBs), was inconclusive due to the variability in the methods of determination, and the reporting of results.

2.1.1 Cohort studies

Table 2.1 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-02-Table2.1.pdf) summarizes 12 cohort studies, published since the last *IARC Monograph*, that evaluate the risk of HCC among individuals who were infected with HBV. Of these, the majority of studies ($n = 7$) were conducted in Asia (Chang *et al.*, 1994; Lu *et al.*, 1998; Evans *et al.*, 2002; Yang *et al.*, 2002; Wang *et al.*, 2003a; Tanaka *et al.*, 2004; Gwack *et al.*, 2007), followed by two studies in Europe (Crook *et al.*, 2003; Ribes *et al.*, 2006) and one study each from Africa (Evans *et al.*, 1998), America (Nomura *et al.*, 1996), and Australia (Amin *et al.*, 2006). Just as the locations of the studies spread across the globe, the study populations and their size, length of follow-up, and study methodologies vary widely.

In these studies, the cohorts consisted of general populations of both genders (Chang *et al.*, 1994; Nomura *et al.*, 1996; Lu *et al.*, 1998; Evans *et al.*, 2002; Yang *et al.*, 2002; Wang *et al.*, 2003a; Gwack *et al.*, 2007), Army recruits (Evans *et al.*, 1998), blood donors (Crook *et al.*, 2003; Tanaka *et al.*, 2004; Ribes *et al.*, 2006), and newly infected people notified to the Australian State Health Department (Amin *et al.*, 2006). The average length of follow-up was as short as 4 years (Amin *et al.*, 2006), and as long as 22 years (Crook *et al.*, 2003). Most studies used an HBsAg-seronegative cohort as a comparison group; a few studies used the general population as a reference cohort (Amin *et al.*, 2006; Crook *et al.*, 2003). Some studies collected information on several other known and potential risk factors for HCC and entered them into a stratified or a multivariate analysis (Chang *et al.*, 1994; Evans *et al.*, 2002; Yang *et al.*, 2002; Wang *et al.*, 2003a; Gwack *et al.*, 2007). In these studies, the risk of HCC was still significantly associated with chronic HBV infection with adjustment for the presence of anti-HCV, cigarette smoking, alcohol drinking, or serum glucose level.

In the cohort studies reviewed, the relative risks ranged from 9.6 (95%CI: 6.0–15.2) (Yang *et al.*, 2002) to as high as 74 (95%CI: 45–121) (Tanaka *et al.*, 2004). The relative risk was found to be even higher, as high as 161 (95%CI: 46–557), if an individual was co-infected with HCV (Tanaka *et al.*, 2004).

A second group of cohort studies included the individuals who had pre-existing liver disease. As pointed out in the previous *IARC Monograph* (IARC, 1994), these studies are difficult to interpret because the causes of liver disease other than HBV infection may also be associated with an increased risk for HCC, leading to an attenuation of the estimated relative risk associated with HBV (Benvegnù *et al.*, 1994, 2001, 2004; Zoli *et al.*, 1996; Tsai *et al.*, 1997; Yu *et al.*, 1997a; del Olmo *et al.*, 1998; Chiaramonte *et al.*, 1999; Di Marco *et al.*, 1999; Yamanaka *et al.*, 2001; Sangiovanni *et al.*, 2004; Ikeda *et al.*, 2005; Mahmood *et al.*, 2005).

2.1.2 Case–control studies

Many case–control studies have been published on the relationship between HCC and HBV infection since the previous *IARC Monograph*. The primary purpose of many of these studies was not to examine HBV infection, but to assess the effect of co-infection by HBV and HCV along with other potential risk factors for HCC. These studies are summarized in Table 2.2 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-02-Table2.2.pdf).

In most studies, tests for HBV markers were performed once and "carriers" were defined as those positive for serum HBsAg at that time. Crude relative risks, as measured by odds ratio and 95% confidence intervals, were calculated by the Working Group when they were not provided by the authors, and wherever the data reported in the original papers allowed it. Studies of clinical series (typically, patients with liver disease) in which cases of HCC were a subgroup but in which there was no specifically defined control group were not included.

As with the cohort studies described above, most of the 31 case–control studies presented in Table 2.2 (on-line) were conducted in either Asia (n = 14) or Europe (n = 9), followed by seven in Africa, and one in the USA. The results from the case–control studies continue to demonstrate a significant association between HBV infection and the risk of HCC in humans. The adjusted odds ratios for HBsAg seropositivity ranged from 1.5–87.4. Eleven studies reported a more than 20-fold increased risk of HCC (Pyong *et al.*, 1994; Park *et al.*, 1995; Shin *et al.*, 1996; Sun *et al.*, 1996; Tsai *et al.*, 1996a; Kew *et al.*, 1997; Zhang *et al.*, 1998; Chiesa *et al.*, 2000; Kuper *et al.*, 2000; Franceschi *et al.*, 2006a; Kumar *et al.*, 2007). The wide range in reported odds ratios from these studies is likely to be explained by the differences in the underlying prevalence of HBV in the communities studied, the numbers of cases and controls studied, the duration of infection, and the type of controls selected for a study.

Potential confounding by other risk factors for HCC, particularly infection with HCV, was addressed in many of these studies. HBV was found to be an independent risk factor for HCC in the presence of other known and potential risk factors such as HCV infection, alcohol drinking, cigarette smoking, and/or diabetes.

(a) Occult hepatitis B infection

All of the epidemiological studies described above and in the previous *IARC Monograph* consider HBsAg seropositivity as a measure of persistent infection with HBV. However, the development of highly sensitive methods for the detection of HBV DNA has made it clear that there are individuals who are viraemic or who have integrated HBV DNA in hepatic tissue, and are negative for HBsAg (Bréchot *et al.*, 2001). Several studies have now reported on the presence of occult HBV associated with HCC from Asia as well as regions with a low prevalence of typical chronic hepatitis B infection.

Yu *et al.* (1997c) presented a re-analysis of a case–control study included in the previous *IARC Monograph*. This was a study of 111 cases of histologically confirmed HCC and 128 controls, all non-Asian, living in Los Angeles County in the USA. They found a 4.7-fold (95%CI: 2.2–9.4) increased risk of HCC among individuals with evidence of a previous HBV infection but who were negative for HBsAg and HBV DNA. In a study of 19 HCC cases conducted at Johns Hopkins Hospital in the USA, it was found that three had HBV DNA present in the liver tissue despite being negative for HBsAg in serum (Kannangai *et al.*, 2004). Squadrito *et al.* (2006) followed a cohort of 134 patients in Italy who had chronic hepatitis but were negative for HBsAg. The analysis of liver biopsy tissue established the presence of HBV DNA in 53 of these subjects. During a median 84-month follow-up, nine new HCC cases were observed, of which eight occurred in the group with occult HBV infection (P = 0.002).

2.1.3 Intervention studies

Vaccination to prevent hepatitis B infection and antiviral treatment of persistent HBV have both been evaluated in relation to their effect on HCC incidence. A decrease in the rate of HCC after vaccination against or treatment

for hepatitis B infection would provide further evidence that HBV is a cause of HCC.

A nationwide hepatitis B vaccination programme was introduced in Taiwan, China in July 1984. The subsequent age-specific incidence of HCC has been studied through the Taiwan (China) National Cancer Registry. In the initial report, the incidence of HCC in children aged 6–14 years declined from 0.70/100000 children during 1981–86 to 0.57 during 1986–90, and to 0.36 during 1990–94 (Chang *et al.*, 1997). Subsequently, it was reported that the decline was primarily in boys born after 1984 whereas the decrease observed in girls was non-significant (Chang *et al.*, 2000). The main problems preventing the eradication of HCC among children were vaccine failure, and a failure to receive hepatitis B immune globulin at birth (Chang *et al.*, 2005). A randomized study of hepatitis B vaccination of children to prevent HCC has been in progress since the mid-80s in Qidong, China (Sun *et al.*, 1991), and The Gambia (The Gambia Hepatitis Study Group, 1987; Viviani *et al.*, 2008). The results are expected in 2015–20 for the Chinese study, and 2017 for the Gambian study.

The treatment of persistent HBV infection with antivirals has been shown to reduce viral load and disease progression. Liaw *et al.* (2004) conducted a randomized placebo-controlled trial of lamivudine alone for 30 months in Asian patients with chronic hepatitis B and advanced liver disease. They reported that HCC developed in 7.4% of 215 subjects in the placebo group, and 3.9% of 436 in the lamivudine group (hazard ratio, 0.49; 95%CI: 0.25–0.99).

2.1.4 HBV/HCV co-infection

No single study has sufficient numbers of co-infected individuals without clinically evident liver disease to provide a reliable estimate of the risk associated with dual infection with HBV and HCV. Two meta-analyses of studies have been carried out to address this difficulty. Donato *et al.* (1998) searched the literature published between 1993–97 for appropriate studies using healthy carriers from cohort studies or healthy controls without chronic liver disease in case–control studies. Studies were only included if they used HBsAg and anti-HCV or HCV RNA for serological markers for HBV and HCV infection, respectively. No cohort studies were suitable to be included in the meta-analysis. A total of 32 case–control studies were included providing 4560 cases and 6988 controls. The summary odds ratio for being HBsAg-positive but anti-HCV/HCV RNA-negative was 20.4 (95%CI: 18.0–23.2), and for HBsAg-positive and anti-HCV/HCV RNA-positive, 135 (95%CI: 79.7–242). The odds ratio for HBsAg-negative and anti-HCV/HCV RNA-positive was 23.6 (20.0–28.1). Significant heterogeneity was found between studies that could not be explained by the generation of the HCV test, geographic area, or type of controls used. However, the results remained consistent in showing that the risk of concurrent infection with HBV and HCV for HCC was more than a sum of the risk from each, but less than a multiplicative product of the two.

In the second meta-analysis, Shi *et al.* (2005) restricted studies to those conducted in China. They searched for all studies between 1979–2004 that used appropriate serological markers of chronic viral infections: HBsAg for HBV and anti-HCV or HCV RNA for HCV infection. They only included studies that compared HCC cases with a control group without chronic liver disease. A total of 32 case–control studies, including 3201 cases and 4005 controls, met the inclusion criteria. Again, there was marked heterogeneity between studies that could not be explained by geographic area or type of control. The summary odds ratio for those HBsAg-positive and anti-HCV/HCV RNA-negative was 15.6 (95%CI: 11.5–21.3), and for HBsAg-positive and anti-HCV/HCV RNA-positive, 35.7 (95%CI: 26.2–48.5). Because the odds ratio for HCV infection alone was 8.1 (95%CI: 5.0–13.0), the result again indicates that the combined effect of

HBV and HCV infections in causing HCC lies between additive and multiplicative.

2.1.5 Hepatitis B viral factors

Viral factors have been shown to influence the risk of HCC in several cohort studies. In particular, HBe antigenaemia (as a surrogate for high viral load) and a high level of HBV DNA markedly increase the subsequent risk of HCC (Yu *et al.*, 2005; Chen *et al.*, 2006a, b; Iloeje *et al.*, 2007; Chan *et al.*, 2008).

The viral genotype also appears to modify the risk of HCC. Some of this data is difficult to interpret because the subjects of the study had chronic liver disease at recruitment, and because of the global variation in viral genotypes. However, Kew *et al.* (2005), in a case–control study of liver cancer, found evidence for an association with genotype A among the Bantu-speaking people of South Africa. A total of 111 individuals with HCC were compared to an equal number of age- and sex-matched asymptomatic chronic carriers of HBV without HCC who were recruited after screening from factories in the Gauteng region of the country. Both cases and controls tested positive for the presence of HBsAg. Among cases, 96 (86.5%) were positive for genotype A compared to 76 (68.5%) of controls, resulting in a relative risk of 4.5 (95%CI: 1.9–10.9). The majority of other remaining subjects were infected with genotype D (8.1% of cases and 23.4% of controls). There was no genotype F detected in these populations.

Livingston *et al.* (2007), in a cohort study of Alaskan native people, examined the viral genotype in 47 patients with HCC and in 1129 subjects without HCC. Genotype F was found in 68% of cases and in 18% of non-HCC subjects. In addition, the median age at diagnosis of HCC was lower for patients with genotype F than patients with other genotypes (22.5 years versus 60 years). In the non-HCC population, 58% had genotype D, 13% genotype A, 7% genotype C, and 4% genotype B. This illustrates the marked differences in genotype prevalence between two countries.

Yang *et al.* (2008) studied the incidence of HCC by genotype in a community-based cohort in Taiwan, China, where the prevalent viral genotypes are B and C. In a multivariable analysis controlling for age, sex, smoking, alcohol and viral load, the relative risk of HCC for genotype C was 1.8 (95%CI: 1.2–2.6) compared to genotype B.

Several studies have also identified an increased risk of HCC associated with mutation in the core promoter sequence of the virus (e.g. Kao *et al.*, 2003; Chen *et al.*, 2007b, c; Chou *et al.*, 2008; Yang *et al.*, 2008).

2.1.6 Factors modifying the risk of HCC associated with hepatitis B

(a) Aflatoxin

Aflatoxin was last reviewed by IARC in Volume 82 (IARC, 2002). It was concluded that a role of aflatoxin in liver cancer etiology, especially among individuals who are carriers of HBsAg, is supported by the overall body of evidence. A key study that examined aflatoxin as a factor in modifying the risk of HCC associated with HBV was a nested case–control study conducted by Qian *et al.* (1994). The odds ratio associated with urinary aflatoxin biomarkers was 3.4 (95%CI: 1.1–10), and for HBsAg positivity alone 7.3 (95%CI: 2.2–24.4). However, when these two risk factors were positive, the odds ratio was 59 (95%CI: 17–212), suggesting multiplicative effect modification. Nonetheless, the previous Working Group commented that "the interpretation of studies was hampered by the difficulties in properly assessing an individual's lifetime exposure to aflatoxins and the difficulties in disentangling the effects of aflatoxins from those of hepatitis infections." Since then, a handful of studies have been published.

Wu *et al.* (2007, 2008) reported results from two different nested case–control studies based

on the same community-based Cancer Screen Program cohort in Taiwan, China. In one, they examined urinary 15-F_{2t}-isoprostane as an indicator of oxidative stress, and showed that it was correlated with urinary aflatoxin–albumin adduct levels. They found that higher levels of this marker increased the risk of HCC particularly in HBsAg-positive subjects. In comparison to those with low urinary 15-F_{2t}-isoprostane and without HBV infection, those with chronic HBV infection and 15-F_{2t}-isoprostane above mean level had an odds ratio of 19.0 (95%CI: 6.7–54.2). In the second study, the association between exposure to polycyclic aromatic hydrocarbons (PAHs) and the risk of HCC was examined. The levels of PAH–albumin adducts were associated with HCC, and appeared to modify the effect of aflatoxin and HBV infection.

Kirk *et al.* (2005a, b) has explored the effects of high dietary exposure to aflatoxins on the risk of HCC in a case–control study in The Gambia, where HBV infection is highly endemic. In the first study, mutations of the *TP53* gene at codon 249 (a mutation associated with aflatoxin exposure) were measured in the plasma of HCC patients and of healthy subjects. The risk of HCC was found to be elevated in those HBsAg-positive (OR, 10.0; 95%CI: 5.2–19.6), in those 249(ser)-positive alone (OR, 13.2; 95%CI: 5.0–35.0), and when both markers were present (OR, 399; 95%CI: 48.6–3270). In the second study, human DNA was analysed for genetic polymorphisms in aflatoxin-metabolizing – and hence activating (GSTM1, GSTT1, HYL1*2) – and DNA-repair (XRCC1) enzymes. Statistically significant associations were found for the null GSTM1 genotype (OR, 2.45; 95%CI: 1.21–4.95), and also for the combined metabolizing enzyme genotypes. The HCC risk was most prominent among the individuals with the highest groundnut consumption (OR, 4.67; 95%CI: 1.45–15.1). These data suggest susceptibility to HCC can be altered by aflatoxin, but do not clearly demonstrate an interaction of aflatoxin with HBV in carcinogenesis.

(b) Alcohol

The association between alcohol consumption and the risk of HCC has been reviewed recently by IARC in Volume 96 (IARC, 2010). The assessment was made difficult by the fact that signs and symptoms of cirrhosis often preceded the cancer, which may have led to a modification of alcohol intake. Thus, in general, any interaction between alcohol and HBV infection is best addressed in cohort studies.

Of the cohort studies reported in that volume, Chang *et al.* (1994) found no effect of alcohol consumption on risk of HCC; therefore it was dropped from the final multivariable model. In the cohort study of 11893 men, Yang *et al.* (2002) found that while alcohol consumption was associated with HCC (RR, 1.5; 95%CI: 1.0–2.3), when individuals who were positive for HBsAg were stratified according to alcohol use status, the relative risk for HCC was 11.4 (95%CI: 5.0–26.3) for men who drank alcohol, and 9.7 (95%CI: 5.6–16.9) for men who did not drink alcohol.

In Evans *et al.* (2002), a relative risk of 0.9 (95%CI: 0.8–1.0) was found for alcohol consumption of more than three drinks per week in men in a multivariable model with no interaction with HBsAg-positivity. In women, alcohol consumption had a relative risk of 0.6 (95%CI: 0.3–1.2) in the multivariable model, and again, no interaction with HBsAg positivity was shown. The studies of blood donors by Crook *et al.* (2003) and Tanaka *et al.* (2004) did not have information on alcohol consumption. A recent cohort study in the Republic of Korea specifically assessed the independent effect and an interaction of alcohol intake and HBV infection on the risk of mortality from HCC (Jee *et al.*, 2004). A total of 1283112 men and women free of cancer at baseline were assessed and followed up from 1993–2002. During this time, 3807 deaths from HCC were observed. Heavy alcohol consumption in men was associated with a relative risk for HCC of 1.5 (95%CI: 1.2–2.0), but there was

no interaction between alcohol drinking and HBsAg positivity.

It is worth noting that these cohort findings of no interaction from Asia are contrary to the findings of many case–control studies. For example, Donato *et al.* (1997) found a positive interaction between self-reported history of heavy alcohol consumption and HBV infection. The relative risk for joint exposures (RR, 64.7; 95%CI: 20–210) was greater than the sum of the relative risks for HBsAg-positivity (RR, 9.1; 95%CI: 3.7–22.5), and for alcohol intake alone (RR, 4.2; 95%CI: 2.4–7.4).

(c) Smoking

The cohort studies that examined smoking as a cofactor found modest elevations of the relative risk for HCC, with no evidence of interaction with HBsAg-positivity. Chang *et al.* (1994) found a small but statistically not significant increased risk of development of HCC associated with cigarette smoking (RR, 1.22; 95%CI: 0.55–2.71). The cohort study of Yang *et al.* (2002) found that when HBV-infected men (HBsAg-positive and HBeAg-positive) were stratified by their smoking status, the relative risk for HCC was higher among smokers (RR, 76.9; 95%CI: 39.4–150.3) than non-smokers (RR, 67.0; 95%CI: 26.1–171.7). Although cigarette smoking was associated with an increased risk of HCC (RR, 1.5; 95%CI: 1.0–2.2), no interaction with HBV infection was apparent. Evans *et al.* (2002) found that smoking in men was not associated with an increased risk of HCC, whereas in women, smoking showed a dose–response trend with increasing cigarette consumption: 1–5 cigarettes/day, relative risk 1.5 (95%CI: 0.4–6.3); 6–10/day, 2.0 (95%CI: 0.6–6.5); > 10/day, 4.2 (95%CI: 1.3–13.8). Jee *et al.* (2004) also found that cigarette smoking was associated with an increased relative risk for HCC mortality. However the increase was in male smokers (RR, 1.4; 95%CI: 1.3–1.6), but not in women (RR, 1.1; 95%CI: 0.8–1.7). No interaction was found in either sex with HBsAg positivity.

(d) Metabolic factors

Two of the cohort studies have reported on the effects of obesity (body mass index [BMI] ≥ 30 kg/m^2) and diabetes on the risk of HCC. Chen *et al.* (2008) found that obesity was associated with a 4-fold risk of HCC (RR, 4.13; 95%CI: 1.38–12.4) in those who were anti-HCV-positive but the association was not significant in HBsAg-positive subjects (RR, 1.36; 95%CI: 0.64–2.89).

Diabetes was associated with an increased risk in those positive for HBsAg (RR, 2.27; 95%CI: 1.1–4.7) as well as those positive for anti-HCV (RR, 3.25; 95%CI: 1.20–8.85). In comparison to the referent group of individuals with no chronic HBV and HCV infections, no diabetes, and low BMI (< 30 kg/m^2), for individuals with chronic HBV and HCV infections, diabetes, and obesity (BMI ≥ 30 kg/m^2), the relative risk was as high as 264.7 (95%CI: 35.2–1993). Yu *et al.* (2008) reported that excess weight increased the risk for HCC among HBsAg-positive men in Taiwan, China. In comparison to men with a normal weight, overweight men (BMI, 25–< 30 kg/m^2) had a relative risk for HCC of 1.48 (95%CI: 1.04–2.12), and obese men (BMI ≥ 30 kg/m^2), 1.96 (95%CI: 0.7–5.4).

(e) Human genetics

There have been several studies of human genetic polymorphisms and their effect on the risk of HCC among HBV carriers, e.g. Yu *et al.* (2003). However, there are, as yet, no consistent findings.

2.2 Cancers other than HCC

2.2.1 Cholangiocarcinoma

In the previous *IARC Monograph* (IARC, 1994), two case–control studies were reported showing no association between HBV and cholangiocarcinoma. Since then, many studies have examined this issue further, and these are summarized in Table 2.3 (available at

http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-02-Table2.3.pdf).

In all case–control studies, the carrier status for HBV was determined by the presence of HBsAg in serum. The risk for cholangiocarcinoma was increased in association with HBsAg seropositivity, with estimates of odds ratios ranging from 1.3–8.9. Potential confounding by HCV, liver fluke infection, gallstones, alcohol consumption and cirrhosis appear to have been excluded in studies in which those factors were evaluated. The odds ratios for three studies were statistically significant, whereas odds ratios for four studies were not.

2.2.2 Non-Hodgkin lymphoma

(a) Cohort studies

Five cohort studies were carried out in countries where the prevalence of HBV carrier status is low, and the transmission patterns differ from that of Asia and Africa. Three were conducted in Europe (Crook *et al.*, 2003; Franceschi *et al.*, 2006b; Ribes *et al.*, 2006), and one study each from the USA (Ulcickas Yood *et al.*, 2007), and Australia (Amin *et al.*, 2006). In all five cohorts, the HBV carrier status of individuals was determined by the presence of HBsAg in serum. In recognition of the possibility of confounding by HIV infection, three of five studies addressed the potential effect of co-infection by multivariate analysis or by excluding HIV-infected individuals from the study (Amin *et al.*, 2006; Franceschi *et al.*, 2006b; Ulcickas Yood *et al.*, 2007). The remaining two studies (Crook *et al.*, 2003; Ribes *et al.*, 2006) could not evaluate the potential confounding effect of HIV infection because no HIV-infected individuals were found in the HBsAg-negative group, or this information was not collected. These two studies of blood donors present higher standardized mortality ratios (SMRs) among HBsAg-seropositive individuals: 3.2 (95%CI: 1.2–6.9), and 3.5 (95%CI: 1.7–6.2). The estimates of relative risks among the three studies that controlled for HIV infection was lower than the above two studies, and ranged from 0.62 (95%CI: 0.32–1.20) to 2.8 (95%CI: 1.2–6.8).

See Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-02-Table2.4.pdf.

(b) Case–control studies

Of the nine case–control studies, of variable quality, that reported on the relationship between HBV infection and risk of non-Hodgkin lymphoma, seven studies found a positive association with the odds ratios for HBsAg-seropositivity among non-Hodgkin lymphoma cases varying from 1.8 (95%CI: 1.1–3.1) to 4.1 (95%CI: 1.2–14.4). Potential confounding by HCV and HIV was addressed by exclusion or adjustment during the analysis in those studies that evaluated these factors. A concern was raised that because HBV infection can be reactivated in 14–50% of patients undergoing chemotherapy for non-Hodgkin lymphoma (Coiffier, 2006), some of the positive association observed in case–control studies may be artefactual (Anderson *et al.*, 2008). Only one study provided information sufficient to discern when the viral marker screening was performed (Kim *et al.*, 2002). In this study, laboratory tests for HBsAg, anti-HCV, and anti-HIV were performed on admission or during the first visit to the outpatient clinic, before any treatment including cancer chemotheraphy was administered. The HBsAg carrier status was specifically associated with B-cell non-Hodgkin lymphoma (OR, 4.6; 95%CI: 2.0–10.3), but not with T-cell non-Hodgkin lymphoma (OR, 1.0; 95%CI: 0.2–4.5). The odds ratio was larger against non-cancer controls than other cancer controls (4.6 versus 2.4).

(c) Other

There is very limited information available to evaluate the relationship between HBV infection and the risk of extra-hepatic cancer other than

non-Hodgkin lymphoma. A few studies were specifically conducted for this purpose.

(i) Cancer of the pancreas

Hassan *et al.* (2008) hypothesized that due to the anatomical proximity of the liver to the pancreas, and because the two organs share common blood vessels and ducts, the pancreas may be another potential target organ for hepatitis viruses. The fact that HBsAg was detected in pure pancreatic juice and bile supports the hypothesis. They compared 476 histologically confirmed cases of pancreatic cancer to 879 age-, sex-, and race-matched healthy controls who were genetically unrelated companions of patients at the same cancer centre in Texas, USA. Serum samples were tested for HBsAg, anti-HBc, and anti-HBs. No cases and only one control were positive for HBsAg. However, past exposure to HBV (anti-HBc-positive) with evidence for HBV recovery or immunity (anti-HBs-positive) was significantly associated with an increased risk of pancreatic cancer (OR, 2.3; 95%CI: 1.2–4.3). Past exposure to HBV without evidence of recovery (anti-HBc-positive/anti-HBs-negative) was associated with a greater risk (OR, 4.0; 95%CI: 1.4–11.1). These odds ratios were adjusted for age, sex, race, state of residency, educational level, smoking, diabetes, alcohol, and family history of cancer.

Berrington de Gonzalez *et al.* (2008) reported on pancreatic cancer and the HBsAg status of 631172 men and women who participated in the Korean Cancer Prevention Study (Jee *et al.*, 2004). HBsAg status was not associated with pancreatic cancer risk (RR, 1.13; 95%CI: 0.84–1.52). The interpretation of the result was somewhat limited because the information on HBsAg was only available for 32% of the cohort.

(ii) Hodgkin disease

One case–control study of Hodgkin disease was available for evaluation. Dal Maso *et al.* (2004) studied 62 histologically confirmed incident Hodgkin disease cases, and 504 control patients. The prevalence of HBsAg in the cases was 1.9% (one HBsAg-positive), and 0.9% in controls (four HBsAg-positive), resulting in an adjusted odds ratio of 1.8 (95%CI: 0.1–21.5).

One cohort study of HBV infected individuals listed Hodgkin disease as one of the outcomes, with a standardized incidence ratio (SIR) of 0.8 (95%CI: 0.3–2.1) (Amin *et al.*, 2006).

3. Cancer in Experimental Animals

In this volume, the Working Group decided not to include a separate section on "Cancer in Experimental Animals" in the *Monographs* on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.

4. Other Relevant Data

4.1 Introduction

At a molecular level, the genesis of HBV-induced HCC is a complex, multifaceted, and multistep process with the essential components being a series of genetic or epigenetic changes in the genes that govern cell proliferation and cell death. The precise roles of the virus and the molecular mechanisms involved in hepatocarcinogenesis, how they interact, and the sequence in which they occur in the pathogenesis of HCC remain elusive. However, the available evidence supports the notion that the development of the tumour is the result of a combination of host responses to the presence of the virus and molecular mechanisms that are directly or indirectly induced by the virus.

HBV is a non-cytopathic virus and the hepatic inflammation and injury that occur in acute and chronic hepatitis and cirrhosis are attributed to the immune responses of the host to the presence

of the virus, especially those of class-1-restricted cytotoxic T lymphocytes. A large proportion of HBV-induced HCCs occurs in association with cirrhosis or, less often, chronic hepatitis, suggesting that the underlying chronic necro-inflammatory hepatic disease frequently provides a mitogenic and possibly also a mutagenic environment in which virus-induced genetic changes can lead to hepatocarcinogenesis (Chisari, 2000; Arbuthnot & Kew, 2001). However, the proportion of patients developing HCC following pre-existing cirrhosis seems to vary in different parts of the world. In particular, in regions of high exposure to aflatoxin, the proportion of patients with pre-existing cirrhosis may be significantly lower than in regions in which aflatoxin is not a risk factor (Brechot *et al.*, 2010).

A recent study compared the transcriptome-genotype-phenotype of more than 50 HCCs, and could identify specific patterns for HBV-associated HCCs (Boyault *et al.*, 2007).

4.2 Chronic necro-inflammatory hepatic disease in hepatocarcinogenesis

HBV-induced chronic necro-inflammatory hepatic disease (cirrhosis and chronic hepatitis) is characterized by continuous or intermittent necrosis of hepatocytes, followed by regenerative proliferation. Its central role in hepatocarcinogenesis is supported by the observation that the lifetime risk for developing HCC in chronic HBV carriers with cirrhosis is higher than that in carriers without cirrhosis (Arbuthnot & Kew, 2001).

Hepatocytes are normally in a quiescent state with an extremely low turnover rate, but they react to the loss of liver cells with an extraordinarily vigorous proliferative response. This response is tightly controlled and lasts only until the initial number of hepatocytes is restored; it does not normally lead to cancer (Fausto, 1997, 1999; Overturf *et al.*, 1997). Existing quiescent hepatocytes are responsible for this regenerative cell proliferation, and only uncommonly do hepatic progenitor (oval) cells directly give rise to tumour cells, although it is possible that hepatocytes originating from these cells are at higher risk for oncogenesis than other hepatocytes (Fausto, 1997, 1999).

The proliferation of hepatocytes is regulated by several factors, including nuclear factor-κ-B (NF-κB), transforming growth factor-α (TGF-α), insulin-like growth factor-2 (IGF-2), and hepatocyte growth factor (HGF) (Grisham, 2001). Transcriptional activation of these factors by mediators, such as tumour necrosis factor-α (TNF-α), chemokines, and interleukins released during the inflammatory process regulates proliferation, and has an anti-apoptotic effect through the upregulation of the anti-apoptotic target gene *BCL2* (Grisham, 2001).

With sustained proliferation, at some point and for reasons as yet poorly understood, the regulation of proliferation may become unrestrained, which is an essential step in hepatocarcinogenesis, complicating chronic necro-inflammatory hepatic disease. Unrestrained hepatocyte proliferation, in association with the accumulation over time of several genetic and epigenetic changes, results in the formation of hyperplastic nodules that may progress to dysplastic nodules, and finally to HCC (Fausto, 1999).

By increasing the hepatocyte turnover rate, chronic necro-inflammatory hepatic disease:

- enhances the risk of a cell acquiring critical mutations,
- leads to reactivation of telomerase, and
- may also provides an opportunity for other selective growth advantage of cells to become manifest.

Concurrently with these oncogenic mechanisms, distortion of the lobular architecture of the liver by fibrosis, and nodular regeneration of hepatocytes in cirrhosis modify normal cell-to-cell and cell-to-extracellular matrix interactions,

which may contribute to the loss of cell-growth control, senescence, and apoptosis (Davis & Kresina, 1996).

4.2.1 Virus-induced chronic necro-inflammatory hepatic disease

One way in which mutations can arise is by the generation of reactive oxygen species and/or reactive nitrogen species that induce oxidative/nitrosative stress and DNA damage (Trush & Kensler, 1991; Bartsch & Nair, 2006). Putative mechanisms of free-radical-induced hepatocyte damage and malignant transformation are the mutagenic properties of the free radicals and their effect on lipid peroxidation (Cheeseman, 1993; Bartsch & Nair, 2006). In addition to DNA modifications caused directly by reactive oxygen species and reactive nitrogen species, DNA bases can be modified by lipid peroxidation products such as *trans*-4-hydroxy-2-nonenal (HNE), 4-hydroperoxy-2-nonenal (HPNE), and malondialdehyde (MDA) to form various exocyclic adducts, including malondialdehyde-deoxyguanine (M_1dG), and etheno- and propano-DNA adducts (Bartsch & Nair, 2006).

Oxidative stress and upregulation of inducible nitric oxide synthase (iNOS) has been demonstrated in chronic viral hepatitis (Loguercio & Federico, 2003). A massive increase (up to 90-fold) in the 1,N^6-ethenodeoxyadenosine (εdA) concentration in urine was detected in HBV-infected patients with chronic hepatitis and liver cirrhosis (Bartsch & Nair, 2006). εdA could arise from HBV-induced chronic inflammation, overproducing reactive oxygen species, reactive nitrogen species, and DNA-reactive lipid-peroxidation-derived aldehydes such as HNE (see Fig. 4.1).

4.2.2 Reactivation of telomerase

During the progression of chronic hepatitis to cirrhosis, progressive shortening of telomeres occurs as a consequence of multiple cycles of cell injury, death, and regeneration, and results in the premature senescence of hepatocytes. Telomere-shortening beyond a critical length causes a proliferative block, which becomes manifest as chromosomal instability, end-to-end fusion, and cell death. Hepatocarcinogenesis is characterized by the evolution of clones of hepatocytes with increased telomerase expression and an immortalized phenotype (Farazi *et al.*, 2003).

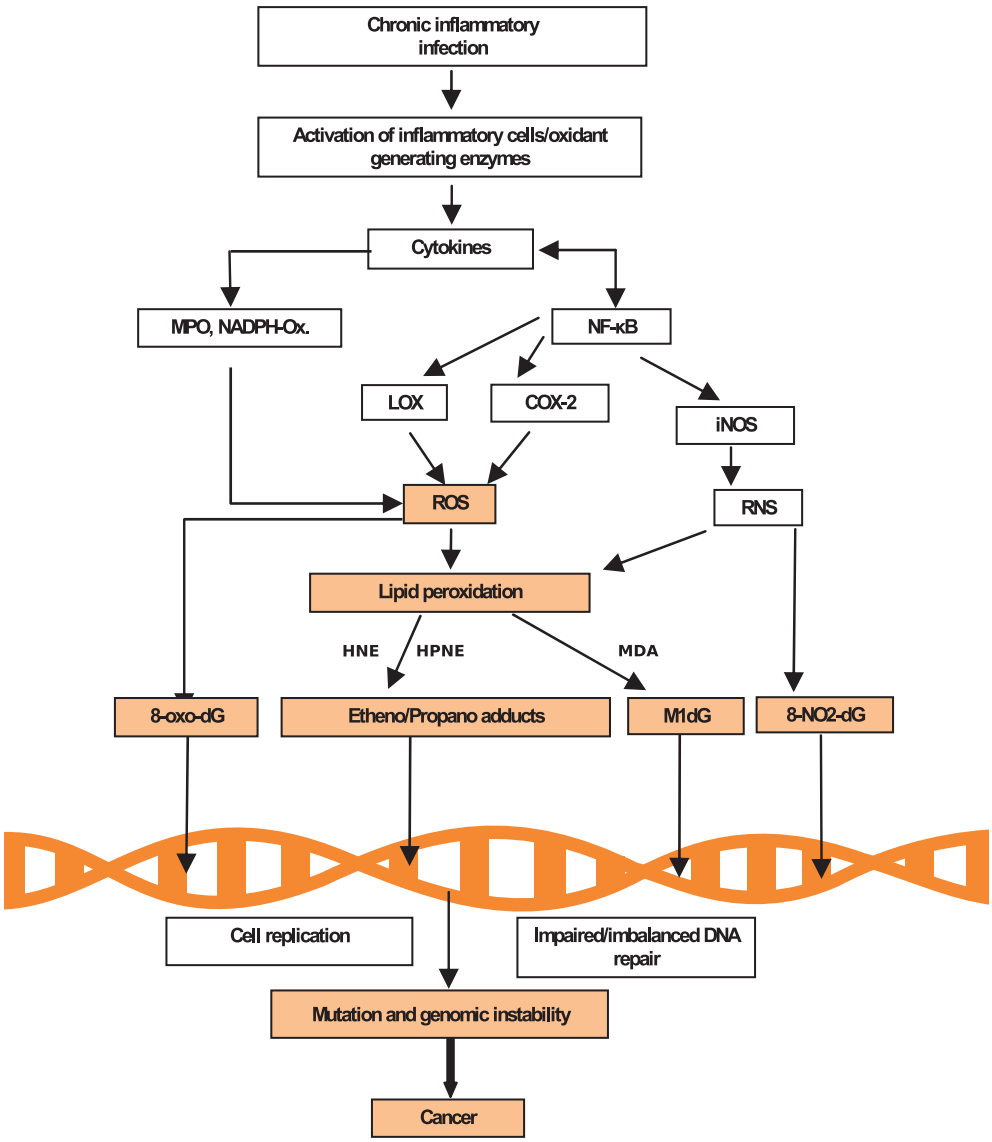
4.3 Direct mechanisms of hepatocarcinogenesis

HBV may also play a direct role in HCC via two major mechanisms. The first mechanism is the integration and mutation of the viral genome into the host cellular DNA, which may result in the altered expression of important cellular genes. The second one is the expression of HBV proteins, which may have a direct effect on cellular functions and in the promotion of malignant transformation (Brechot *et al.*, 2010).

4.3.1 Role of the integration of HBV DNA into the host genome

Although insertion of hepadnaviral DNA into host DNA is not a requirement for viral replication, HBV genome integrations have been reported in over 85–90% of HBV-related HCCs (Bonilla Guerrero & Roberts, 2005). Integration occurs as a result of a recombination event and takes place at one or, far more often, multiple sites (Matsubara & Tokino, 1990; Rogler & Chisari, 1992; Robinson, 1994). Integration is an early event and selective clonal amplification of hepatocytes with unique integration patterns is thought to occur during progression to malignancy (Minami *et al.*, 2005). The integrant may be a single linear sequence of the viral genome

Fig. 4.1 Illustration explaining how chronic infection and inflammatory processes can lead to deregulation of cellular homeostasis and carcinogenesis



ROS, reactive oxygen species; RNS, reactive nitrogen species; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; NADPH-Ox, NADPH oxidase; MPO, myeloperoxidase; LOX, lipoxygenase; HNE, trans-4-hydroxy-2-nonenal; HPNE, 4-hydroperoxy-2-nonenal; MDA, malondialhedyde; M_1dG, malondialdehyde-deoxyguanine; 8-oxo-dG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; 8-NO_2-dG, 8-nitroguanine
With kind permission from Springer Science+Business Media: Langenbecks Arch Surg, Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair, 391, 2006, 499–510, Bartsch H, Nair J, Fig. 1.

(almost always with nucleotides missing from one or both ends), but more often comprises rearranged fragments of viral DNA. Complete and intact viral genomic DNA has been found only rarely in integrants. No two insertions are alike. Linear viral DNA is the preferential form used as an integration substrate (Yang & Summers, 1999).

The exact mechanisms of hepadnaviral DNA integration into cellular DNA are not known, but the evidence available suggests that integration preferentially occurs at sites of double-strand DNA breaks in the cellular DNA (Bill & Summers, 2004). The frequent cell divisions and DNA strand breaks that occur in chronic hepatitis and cirrhosis create opportunities for HBV DNA to be integrated into chromosomal DNA (Dandri *et al.*, 2002). In addition, the existence of preferred topoisomerase-1 cleavage motifs in the vicinity of the DR1 and DR2 sites, may predispose the insertion of HBV DNA into cellular DNA (Wang & Rogler, 1991).

There are several ways in which integrated hepadnaviral DNA may contribute to hepatocarcinogenesis. The insertion of HBV DNA into cellular DNA in human HCC does not occur at specific sites; however, several reports have found integration events near cellular oncogenes or other genes involved in cellular growth (Paterlini-Bréchot *et al.*, 2003; Bonilla Guerrero & Roberts, 2005; Minami *et al.*, 2005). Among the cellular genes with HBV integration events are retinoic acid receptor β, cyclin A2, mevalonate kinase, mcm8, neurotropic tyrosin receptor kinase 2 (NTRK2), IL1R-associated kinase 2 (IRAK2), p42 mitogen-activated protein kinase 1 (p42 MAPK1), telomerase, and others (Dejean *et al.*, 1986; Wang *et al.*, 1990; Paterlini-Bréchot *et al.*, 2003; Bonilla Guerrero & Roberts, 2005; Minami *et al.*, 2005). These studies suggest that the presence of an integrated HBV genome could lead to the inappropriate activation of targeted cellular genes, and in these cases, provide a mechanism for HBV-mediated carcinogenesis.

In the case of woodchuck hepatitis virus (WHV), the integration of the viral genome in or near c-MYC or N-MYC proto-oncogenes in 50% of infected animals considerably enhances the transcriptional activity of the corresponding cellular promoters (Fourel *et al.*, 1990; Tennant *et al.*, 2004). This observation provides strong evidence for a direct role of WHV in hepatocarcinogenesis. However, given that no human cellular gene appears to be targeted with a similar frequency by HBV, it is currently unclear to what extent this mechanism applies to human liver cancer.

Integration of HBV DNA into cellular DNA may also induce structural changes in the flanking DNA sequences. These are highly varied, include small and large deletions, translocations, duplications, or amplification of chromosomal sequences (Matsubara & Tokino, 1990; Takada *et al.*, 1990; Buendia, 2000; Laurent-Puig *et al.*, 2001). Such changes occur more frequently in HBV-related HCC than in HCC attributable to other causes (Laurent-Puig *et al.*, 2001).

4.3.2 Role of HBV proteins

Transcriptional activation by HBV proteins of cellular genes distant from the site of integration (transactivation) that influence cellular proliferation and differentiation or apoptosis is a more frequent, and probably more important, mechanism of inherent hepatocarcinogenesis (Buendia, 2000). This effect is mediated through signal transduction pathways. Two HBV proteins, HBx and PreS/S (when 3′ truncated during or after integration), have been shown to have indirect transactivating capability, and have been implicated in the development of HCC by this means (Murakami, 1999; Feitelson, 2006).

(a) Hepatitis B virus x protein (HBx)

The smallest HBV protein (16.5 kDa), HBx, expressed both in the cytoplasm and the nucleus, is essential for viral replication (Murakami, 1999; Feitelson, 2006).

Because the gene is close to the preferred integration sites of HBV, it is the region of the genome most often included in integrants (Paterlini *et al.*, 1995), and a selective accumulation of HBx gene transcripts has been reported in HBV-related HCC. Antibodies against HBx have been demonstrated in the sera of chronic carriers, which confirms the expression of the viral protein (Pál *et al.*, 2006).

Integrated HBx, even when truncated and mutated, may still retain some of its functions. Alternatively, mutation and/or truncation may activate specific functions of HBx (Schlüter *et al.*, 1994). Evidence that HBx is contributing to HCC comes in part from knockdown experiments in which reduction in the levels of HBx leads to growth suppression (Chan & Ng, 2006; Cheng *et al.*, 2007). In addition, no HCC related to avian hepadnaviruses, which are devoid of the X ORF, have been reported (Murakami, 1999).

HBx does not contain any structural motifs that indicate a capacity to bind DNA directly, and functions through protein-to-protein interaction. HBx activates transcription from various HBV promoters, other viral promoters, and from the promoters of a large number of cellular genes including oncogenes, cytokines, growth factors, and several genes involved in cell-cycle control and progression, DNA repair, apoptotic cell death, cellular adhesion, and angiogenesis (Murakami, 1999; Feitelson, 2006; Table 4.1).

A wide variety of cis-elements have been shown to be responsive to HBx which includes binding sites for AP-1, AP-2, NF-κB, SRF, c/EBP, Ets, ATF1, and CREB (Table 4.1).

Transcriptional regulation by HBx may occur through direct interaction with transcription factors in the nucleus, like shown for ATF-2, CREB, Oct-1, p53, bZIP, and other components of the basal transcription machinery (Table 4.2).

HBx transactivation may also occur by modulating cell-signalling pathways within the cytoplasm (Table 4.3). NF-κB-signalling that mediates cellular stress responses that control the expression of several acute-phase response proteins, cytokines, and adhesion molecules is among the pathways modulated by HBx though the activation of "Mitogen Activated Protein Kinase" (MAPK) pathways (Benhenda *et al.*, 2009).

HBx has also been shown to inhibit the activity of some serine protease inhibitors and components of the proteasome complex, specifically PSMA7 (Zhang *et al.*, 2000), and might thus modulate the turnover of certain cellular proteins involved in transcription or regulation of cell-cycle progression, or both.

Among the cellular proteins whose functions are known to be perturbed by HBx protein is the tumour-suppressor p53. The p53 protein maintains chromosomal integrity by arresting the cell cycle in G_1, regulating the DNA damage control responses, and regulating the induction of apoptosis and/or senescence (Shimamura & Fisher, 1996). *In vitro* HBx expression studies have shown that HBx protein binds to specific sequences in the *C*-terminal end of p53, preventing its entry into the nucleus, and abrogating its sequence-specific DNA-binding and transcriptional activity (Elmore *et al.*, 1997; Takada *et al.*, 1997).

"Phosphatase and Tensin homology deleted on chromosome 10" (PTEN) is another important tumour-suppressor which has been shown to be a transcriptional target of p53 (Stambolic *et al.*, 2001). Inactivation of both p53 and PTEN proteins by HBx protein results in increased levels of hypoxia-induced factor-1α (HF1-α) and vascular endothelial growth factor (VEGF), both of which are important for the survival and neovascularization of early-stage tumours (Huang & Kontos, 2002).

Table 4.1 Some targets of transactivation by HBx

Gene product	Binding factor involved
Interleukin-8	NF-IL6 and NF-κB-like
HLA-DR	nd
ICAM-1	nd
EGF receptor	
Alpha-fetoprotein	c/EBP site
MDR	
TGFα	[a]AP-2
TGFβ	Egr1 (Ets family) HBx interaction with Egr1
Interleukin-6	NF-κB
TNFα	Proximal promoter
C-FOS	nd
C-JUN	AP-1
C-MYC	nd
TBP	nd

[a] reference Kim & Rho (2002)
nd, not determined
Adapted from Murakami (1999)

HBx protein may impair DNA-repair mechanisms by several means. Besides its potential inhibition of p53-dependent DNA repair, HBx can repress the transcription of two components of the repair factor TFIIH – XPB and XPD (Jaitovich-Groisman *et al.*, 2001). The protein also directly interferes with DNA repair by forming a complex with the DNA-repair protein, HBx-associated protein (XAP-1), which normally binds to damaged DNA in the first step of nucleotide excision repair (Becker *et al.*, 1998). Recent evidence shows that HBx binds the UV-damaged DNA binding protein 1 (DDB1), a protein involved in DNA repair and cell-cycle regulation. This interaction leads to interference with *S*-phase progression and induces lagging chromosomes during mitosis. Consequently, HBx may exert deleterious activities in dividing, but not quiescent, hepatoma cells (Martin-Lluesma *et al.*, 2008).

In addition to its inhibitory effects on p53-induced apoptosis, HBx protein inhibits caspase-3-dependent apoptosis (Gottlob *et al.*, 1998). Conversely, HBx may also sensitize cells to programmed cell death induced by TNF-α, an effect mediated by prolonged stimulation by N-*MYC* transcription and the stress-mediated MAPK pathway (Su & Schneider, 1997).

(b) Hepatitis B virus 3′ truncated PreS/S proteins

Like the *HBx* gene, the *PreS/S* gene is frequently included in HBV DNA integrants in patients with HCC. When 3′ truncated during or after integration, the gene has transactivating properties and might contribute to oncogenesis (Schlüter *et al.*, 1994). The truncated medium surface protein is exclusively cytoplasmic in location and has pleotropic effects on gene transcription. Its transactivating effects are mediated by modulating protein kinase C (PKC) signal transduction and interaction with several transcription factors such as NF-κB and AP-1 (Lauer *et al.*, 1994; Hildt *et al.*, 1996). Potentially oncogenic transcriptional effects include the stimulation of promoter sequences of c-*MYC*, c-*FOS*, and c-HA-*RAS* oncogenes, and the inflammation-associated cytokine, IL-6 (Kekulé *et al.*, 1990; Meyer *et al.*, 1992; Lauer *et al.*, 1994). Mutated

Table 4.2 Some HBx-interacting cellular proteins

Protein	Function
Transcription factors	
bZip family	
ATF, CREB/ATF-2	
ATF3, NF-IL6	
Chop10, ICER IIγ	
Oct1	
Egr1	
P53	Tumour suppressor Transcription factor
General transcription factors	
TBP	TATA binding
RPB5	A common subunit of pol I, II & III
TFIIB	Initiation factor
DNA repair	
TFIIH	Complex necessary for initiation and elongation
ERCC2	
ERCC3	
UVDDB1 (XAP1)	DNA repair
Protease subunit	
XAPC7	Proteasome α-subunit
Other pX-binding proteins	
XAP2	AhR ligand-binding subunit ARA9 family of BREF-2
XAP3	PKC Binding protein
XIP	Novel, ubiquitous
P55sen	In family of EGF-like proteins

Adapted from Murakami (1999)

PreS protein may induce endoplasmic reticulum stress (Wang *et al.*, 2003b), which in turn stimulates the expression of cyclooxygenase-2 through activation of NF-κB and P38-MAPK (Hung *et al.*, 2004). In transgenic mice that overproduce in hepatocytes the large envelope protein of HBV, this protein accumulated in the endoplasmic reticulum. This led to cytopathic effects that contributed to a progressive disease culminating in liver cancer (Chisari *et al.*, 1989). The relevance of this animal model to HBV-associated HCC in human remains unclear.

4.4 Epigenetic mechanisms

Methylation of CpG islands of tumour-related genes is an early and frequent event in the multistep process of hepatocarcinogenesis, with an increasing number of tumour-suppressor genes being affected by epigenetic silencing (Lee *et al.*, 2003b; Oh *et al.*, 2007). There is evidence that genome-wide methylation patterns may vary according to HCC etiology (Hernandez-Vargas *et al.*, 2010). Deregulated expression of DNA methyltranferases by HBx may contribute to the epigenetic modulation of cellular genes involved in cell cycle (Kanai *et al.*, 1997; Lee *et al.*, 2003b; Oh *et al.*, 2007; Park *et al.*, 2007; Su *et al.*, 2007a).

Table 4.3 Some reported interactions of the HBx viral protein with major cellular signal-transduction pathways

Signalling pathways	Reported HBx interactions
p53/PTEN	- binds to and inactivates p53 - blocks PTEN expression via binding p53
pRB	- promotes hyperphosphorylation (inactivation) of pRB - promotes pRB expression
$p21^{WAF1/CIP1}$	- suppresses *p21* promoter via binding to wild type p53 or $p55^{sen}$
MYC	- stimulates the *c-MYC* promoter
RAS/RAF/MAPK	- stimulates RAS/RAF/MAPK signalling
E-Cadherin	- stimulates methylation of the *E-cadherin* promoter
IGFR1	- upregulates IGFR1 - inactivates p53
TGFβ1	- upregulates TGFβ1 and TGFβ1 signalling resulting in loss of sensitivity of cells to TGFβ1.
JAK/STAT	- activates JAK

Adapted from Feitelson (2006)

4.5 Other major risk factors in hepatocarcinogenesis

4.5.1 HCV infection

Dual infection with HBV and HCV is common, and is associated with more severe chronic hepatic parenchymal disease and an increased frequency and a younger age of development of HCC than occurs with either virus alone (Kaklamani *et al.*, 1991; Kew, 2006). Understanding the nature of the synergistic interaction between the two viruses in hepatocarcinogenesis will have to wait until a clearer understanding of the mechanisms involved in HCC induced by either virus alone is attained. Moreover, the replicative dominance of one virus over the other and the effect that this may have on the progression of liver disease and the development of HCC remains a matter of debate (Zarski *et al.*, 1998; Kew, 2006). Nevertheless, several possible mechanisms for the synergistic hepatocarcinogenic interaction between the two viruses are suggested by currently available information, and the major players appear to be HBx protein, HCV core, and NS5a proteins.

In Africa and Asia, chronic infection with HBV that gives rise to HCC is predominantly acquired very early in life, whereas chronic HCV infection in industrialized countries is largely acquired much later in life. It is likely that HCV infection is superimposed on a long-standing HBV infection in the great majority of patients with dual infection in Asia and Africa; whereas in developed countries, it is probable that the two infections are obtained either at the same time or within a relatively short interval. These differences may influence the mechanisms involved in hepatocarcinogenesis in patients co-infected with HBV and HCV (Kew, 2006).

Dual infection with HBV and HCV results in a higher incidence of cirrhosis than with either virus alone (Tsai *et al.*, 1996b), so the possible mechanisms implicated in malignant transformations complicating chronic necro-inflammatory hepatic disease are even more likely to be applicable with co-infection. Apart from the importance of hepatocyte necrosis and regeneration in generating oxidative damage, the HCV core and NS5a proteins have been reported to directly generate reactive oxygen species (Gong *et al.*, 2001; Okuda *et al.*, 2002).

HBx and HCV core proteins can additively repress transcription of the *p21* gene (Han *et al.*, 2002). Because the tumour-suppressor protein p21 is a universal inhibitor of cyclin-CDK complexes and proliferating cell nuclear antigen (PCNA) and hence DNA replication by inducing cell-cycle arrest at the G1-S checkpoint, the combined repression of *p21* by HBx and HCV core proteins may result in an additive growth stimulation of hepatocytes (Han *et al.*, 2002).

A specific mutation, T1936C, has been reported in the proximal core region of HBV that may be involved in the accelerated progression to HCC in co-infected patients (De Mitri *et al.*, 2006).

The relevance of potential interactions between the two viruses in human HCC is supported by the recent report by Rodríguez-Iñigo *et al.* (2005), who demonstrated by in-situ hybridization that HCV and HBV can coexist in the same hepatocyte in liver biopsy samples from patients with chronic HCV infection with occult HBV infection.

4.5.2 Aflatoxin B_1

Early evidence of hepatocarcinogenic synergism between hepadnavirus infection and dietary exposure to the fungal toxin, aflatoxin B_1 (AFB_1) was provided by experiments in transgenic HBV-mice and in woodchucks infected with WHV. This evidence was confirmed in ecological studies wherein the majority of which, the increased risk was multiplicative (reviewed in Kew, 2003; Gouas *et al.*, 2009; Wild & Montesano, 2009).

Several mechanisms have been suggested to explain the synergism. The first is that the cytochrome P450s that convert the AFB_1 parent molecule to the highly reactive AFB_1-8,9-*exo*-epoxide may be induced by either chronic hepatitis caused by HBV infection or the presence of the virus itself (Kirby *et al.*, 1994). A recent study shows that this epoxide forms preferential adducts in DNA at G bases located in sequence in a similar context to the one of codon 249 in TP53 (Besaratinia *et al.*, 2009).

Another way in which hepatocytes may be sensitized to the carcinogenic effects of AFB_1 is by the decreased activity of the phase II detoxification enzymes, glutathione-*S*-transferase (GST) and EPHX (McGlynn *et al.*, 1995). In human liver, GST activity is lower in the presence of HBV DNA (Zhou *et al.*, 1997). This suggests that the ability of hepatocytes to detoxify chemical carcinogens may be compromised in HBV-infected individuals.

The accelerated hepatocyte proliferation caused by HBV-induced chronic necro-inflammatory hepatic disease increases the likelihood of AFB_1-induced mutations (including 249^{ser} TP53 mutation) being formed, and the subsequent clonal expansion of hepatocytes containing these mutations (Kew, 2003). It also results in the generation of reactive oxygen and nitrogen intermediates, which also induce these mutations (Kew, 2003). AFB_1–DNA adducts, which are normally repaired by the nucleotide excision repair pathway, may persist because of the interference of HBx protein with this pathway (Jia *et al.*, 1999).

4.6 Role of HBV in other cancers

4.6.1 B-cell lymphoma

At the time of writing, no mechanisms are known that might explain the noted limited association between HBV and B-cell lymphoma.

4.6.2 Cholangiocarcinoma

At the time of writing, no mechanisms are known that might explain the noted limited association between HBV and cholangiocarcinoma.

4.7 Synthesis

There is strong evidence to support an indirect role for HBV in hepatocarcinogenesis resulting from chronic necro-inflammatory hepatic disease (cirrhosis), as well as moderate evidence for a direct role largely associated with HBx.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with HBV. Chronic infection with HBV causes hepatocellular carcinoma. Also, positive associations have been observed between chronic infection with HBV and cholangiocarcinoma and non-Hodgkin lymphoma.

Chronic infection with HBV is *carcinogenic to humans (Group 1).*

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