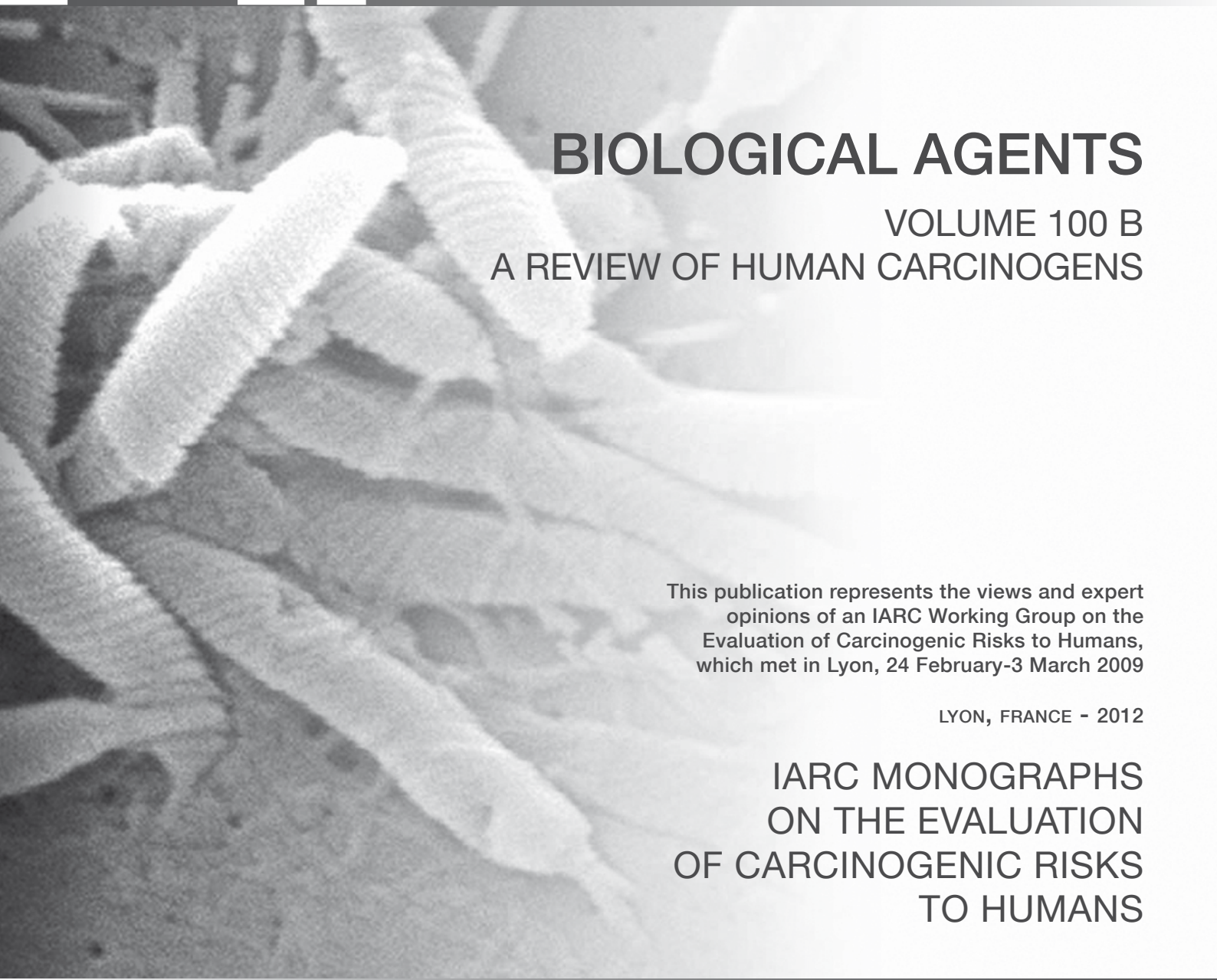
IARC MONOGRAPHS



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

LYON, FRANCE - 2012

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

International Agency for Research on Cancer
World Health Organization

HEPATITIS C VIRUS

The hepatitis C 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

In the 1970s and 1980s, serological tests developed for hepatitis A and B viruses (HAV, HBV) indicated that most transfusion-associated hepatitis was not caused by either HAV or HBV, and were therefore named non-A, non-B hepatitis (NANBH). After detection of the first NANBH-specific clone, the entire viral genome of the now termed hepatitis C virus (HCV) was sequenced, and based on its structural and functional organization, HCV was classified into the family of the *Flaviviridae*, where it forms its own genus '*hepacivirus*' (Choo *et al.*, 1989; Kuo *et al.*, 1989).

At least six major viral genotypes (1 to 6) have been identified (Simmonds *et al.*, 2005). Viral genotypes have specific geographic distribution. The determination of the viral genotype is important for transmission studies, and has major therapeutic implications. Patients infected with genotype 1 have a significantly lower rate of response to antiviral therapy compared to other genotypes (Manns *et al.*, 2007).

Genotypes display differences in nucleotide sequences below 30–35%, and within a genotype genomes are allocated into different subtypes if their sequences differ by over 20–25%. Furthermore, viral isolate(s) present in an infected individual can mutate into quasi-species (Simmonds *et al.*, 2005; Xu *et al.*, 2008).

1.1.2 Structure of the virion

In line with other members of the *Flaviviridae*, HCV consists of an enveloped nucleocapsid that assembles intracellularly in close conjunction with membranes derived from the endoplasmic reticulum (Moradpour *et al.*, 2007). Released HCV particles are about 40–70 nm in diameter, but the morphology of particles detected in the serum of patients may be heterogeneous due to the association with immunoglobulins or very low density lipoproteins (vLDL) (André *et al.*, 2005).

1.1.3 Structure of the viral genome

The HCV genome is a single-stranded, positive sense RNA of approximately 9.6 kb, and is represented in Fig. 1.1. It contains short non-coding regions (NCRs) at each end that encompass the coding sequence of a polyprotein. The 5′ NCR, a well conserved 341nt sequence element which

forms into a complex secondary structure, is needed for efficient RNA replication and drives cap-independent translation of a single large open reading frame (ORF) that encodes approximately 3000 residues (Moradpour *et al.*, 2007). The HCV 3′ NCR consists of a short variable domain of about 40nt and a polyuridine/polypyrimidine tract, followed by a highly conserved domain of 98nt that is essential for RNA replication (Tanaka *et al.*, 1995). The *N*-terminal region of the polyprotein encodes the structural proteins including the nucleocapsid protein (core) and two envelope glycoproteins (E1 and E2) that form the viral particle, followed by several non-structural proteins, designated as NS2 to NS5B (Fig. 1.1). The *C*-terminal regions of the core and envelope proteins contain signal sequences, and are cleaved in the endoplasmic reticulum by host signal peptidase and signal peptide peptidase (Yasui *et al.*, 1998; McLauchlan *et al.*, 2002; Okamoto *et al.*, 2004). Alternative cleavage sites at the *C*-terminal of E2 yield the viroporin p7. The NS2/NS3 junction is cleaved in *cis* by metalloproteinase activity. The remaining cleavages are carried out by the NS3 serine protease, which requires NS4A as a cofactor. Besides participating in polyprotein processing, NS2 plays important roles in viral assembly (Moradpour *et al.*, 2007; Jirasko *et al.*, 2008). The non-structural genes *NS3*, *NS4A*, *NS4B*, *NS5A* and *NS5B* have diverse functions (see Section 4), and are all required for RNA replication (Lindenbach *et al.*, 2007; Moradpour *et al.*, 2007). *NS5B*, the viral replicase, lacks proofreading activity. This lack of proofreading in the context of a very high viral production rate, which has been estimated to be as much as 10^{12} virions per day in infected patients, is thought to be responsible for the high genetic variability of HCV (Moradpour *et al.*, 2007). Thus, HCV has been classified into six genotypes and several subtypes based on sequence similarities. In addition, the HCV genome is prone to acquiring mutations that lead to the presence of quasi-species in infected patients (Xu *et al.*, 2008).

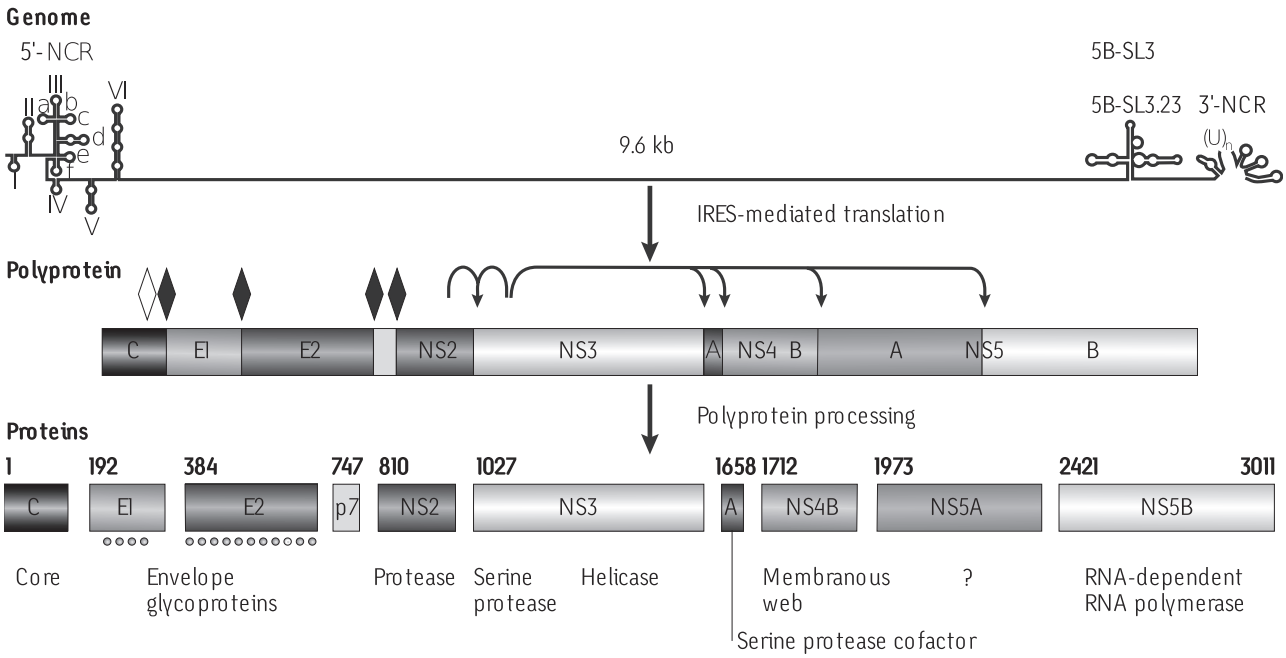
1.1.4 Host range

HCV is hepatotropic, and only man and higher primates such as chimpanzees have been, until recently, receptive to HCV infection and disease. Later, it was shown that the marmoset as well as tupaias, which are members of the tree shrew genus, are also susceptible to HCV infection (Shimizu *et al.*, 1998; Sung *et al.*, 2003; Pachiadakis *et al.*, 2005).

1.1.5 Tissue target

While HCV RNA has been unequivocally detected in the hepatocytes of liver biopsies of chronically infected patients and chimpanzees, the HCV genome has also been suggested to replicate in cells of lymphoid origin and dendritic cells (Shimizu *et al.*, 1998; Sung *et al.*, 2003; Pachiadakis *et al.*, 2005), but this observation remains to be substantiated. Whether the tropism of HCV is limited to hepatocytes at the level of cell entry and/or replication via the association of HCV with lipoproteins and/or by other factors remains unclear. Expression patterns of all cell entry factors isolated to date are either ubiquitous or not unique to hepatocytes. This includes the lipoprotein receptors scavenger receptor B1 (SR-B1), and the low-density lipoprotein receptor (LDLr), which may mediate indirect uptake of HCV via HCV-associated lipoproteins (Moradpour *et al.*, 2007). The replication of HCV seems mostly restricted to hepatocytes, and even though HCV replicons have been shown to amplify in cell lines of non-hepatic origin, the levels of replication reported are significantly lower than those observed in cell lines of hepatic origin (Zhu *et al.*, 2003; Ali *et al.*, 2004; Chang *et al.*, 2006).

Fig. 1.1 The HCV genome structure, viral polyprotein expression and processing into viral proteins



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1.1.6 Life cycle, replication, and regulation of gene expression

Virus-binding to the cell surface and cell entry may involve the LDLr, glycosaminoglycans, SR-B1, the tetraspanin CD81, and the tight junction factors Claudin-1 and Occludin (Evans *et al.*, 2007; Lindenbach *et al.*, 2007; Moradpour *et al.*, 2007; Ploss *et al.*, 2009;). The tight junction factors are thought to act at late stages of cell entry, and their involvement in HCV cell entry suggests that the state of polarization of hepatocytes is likely to be important for the cell entry process (Evans *et al.*, 2007). Internalization occurs via clathrin-coated vesicles, and their acidification induces the fusion machinery of the HCV glycoproteins. Little is known about the uncoating process and the initial events that allow the assembly of replication complexes, IRES-mediated replication, polyprotein processing, and virion assembly. RNA replication occurs in membrane-like webs that are formed at the endoplasmic reticulum. The assembly and secretion process is thought to occur in tight relation with the vLDL biosynthesis machinery, which may explain the possible association of secreted HCV particles with vLDL (André *et al.*, 2005; Nielsen *et al.*, 2006; Miyanari *et al.*, 2007).

1.1.7 Diagnosis of HCV infection

The diagnosis of HCV infection relies on laboratory tests which include: 1) anti-HCV antibody detection assays relying on third-generation enzyme-linked immunosorbent assays (ELISAs) whose sensitivity and specificity have been demonstrated; 2) viral genome detection assays mainly relying on real time Polymerase

Chain Reaction (PCR) technologies allowing the quantification of the viral genome; 3) genotyping assays to determine viral genotypes that allow the prediction of treatment outcome, and the determination of treatment duration (Zoulim, 2006).

The detection of anti-HCV antibodies in plasma or serum is based on the use of third-generation enzyme immunoassays (EIAs) that detect antibodies directed against various HCV epitopes. Recombinant antigens are used to capture circulating anti-HCV antibodies onto the wells of microtitre plates, microbeads, or specific holders adapted to closed automated devices. The specificity of third-generation EIAs for anti-HCV is greater than 99% and has been improved by the addition of viral epitopes that are recognized by antibodies present in the serum of infected patients (Colin *et al.*, 2001). Their sensitivity is more difficult to determine, given the lack of a gold standard method, but it is excellent in HCV-infected immunocompetent patients. EIAs can be fully automated, and are well adapted to large volume testing. Immunoblot tests are nowadays clinically obsolete given the good performance of third-generation anti-HCV EIAs (Colin *et al.*, 2001).

1.2 Epidemiology of infection

1.2.1 Prevalence and geographic distribution

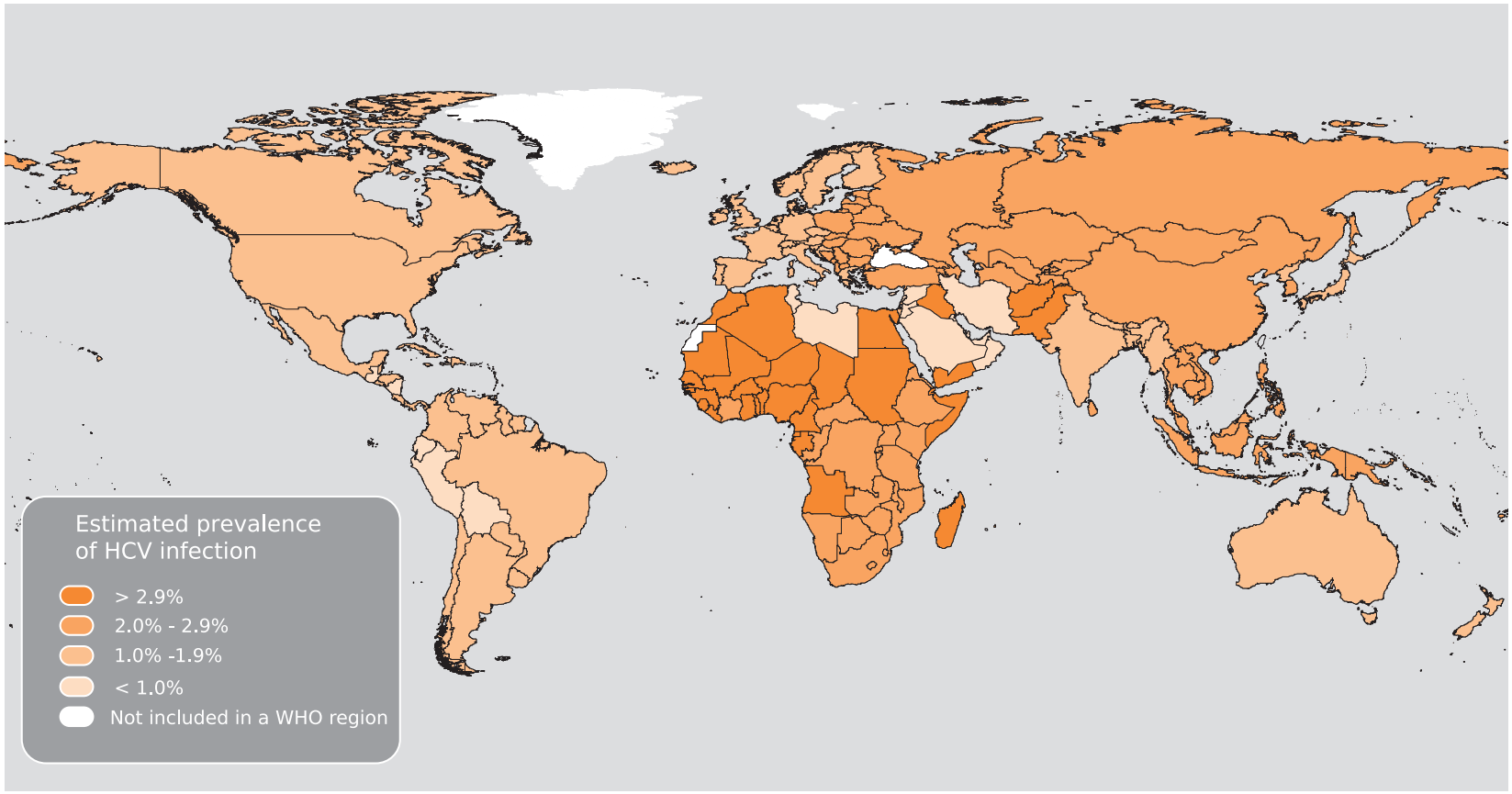
The estimated prevalence of HCV infection worldwide is 2.2%. Region-specific estimates range from < 1.0% in northern Europe to > 3% in northern Africa (Fig. 1.2; Alter, 2007). High prevalences of HCV (≥ 10%) were found in some areas of Italy and Japan and, most notably, in Egypt (15–20%) following mass injection treatment for schistosomiasis. More recently, high HCV prevalences have been reported in some Asian areas, notably in Pakistan (Ahmad, 2004), and the People's Republic of China (Zhang *et al.*, 2005). Within Europe, the highest prevalence rate of HCV infection was reported in southern Italy where 7.5% of the general population and up to 23.2% of those aged 65 years old or older who were randomly selected were HCV-infected (Fusco *et al.*, 2008). The lowest prevalence (0.01–0.1%) was reported for the United Kingdom and Scandinavia.

1.2.2 Transmission and risk factors for infection

HCV can be transmitted by transfusion of blood and blood products, transplantation of solid organs from infected donors, injection drug abuse, unsafe therapeutic injections, and occupational exposure to blood (primarily contaminated needles) (Alter, 2007). Transfusion-associated HCV infection was an important source of infection before HCV testing of blood donors was introduced in the early 1990s. Since then, transfusion-associated HCV infection has been virtually eliminated in those countries where routine HCV-testing has been implemented (Safe Injection Global Network (SIGN), 2001). Iatrogenic HCV transmission through unsafe (therapeutic) injections has sustained substantial epidemics of the infection in Japan, Italy in the 1940s and Egypt in the 1950s, and it is currently very frequent in low-resource countries (Ahmad, 2004; Raza *et al.*, 2007) where disposable needles tend to be re-used, and injections tend to be preferred to the oral route for the administration of common treatments. It has been estimated that approximately 2 million HCV infections are caused annually by contaminated health-care-related injections. Injection drug use accounts for most of the newly acquired infections in developed countries. The incidence rate among new drug injectors has been observed to range from 9 to 30% per 100 person–years (Des Jarlais *et al.*, 2003).

HCV is less efficiently transmitted by occupational, perinatal and high-risk sexual exposures compared to those involving large or repeated percutaneous exposures to blood (Alter, 2007).

Fig. 1.2 Estimated prevalence of HCV infection by region



Adapted from Alter (2007), data source World Health Organization

The rate of transmission after an accidental needle-stick injury involving HCV-positive blood ranges from 0–10% (Hernandez *et al.*, 1992; Mitsui *et al.*, 1992). The rate of perinatal HCV transmission is 4–7% and occurs only when HCV RNA is detectable in the maternal serum at delivery. There has been no difference in the rate of HCV transmission between vaginal delivery, caesarian section or breastfeeding. However, co-infection with HIV increases the rate of transmission 4–5-fold. The extent to which HCV is transmitted by sexual activity is very controversial (Alter, 2007). Although case–control studies of acute hepatitis C have identified sex with an infected partner or with multiple partners as independent risk factors for acquiring the disease, in long-term monogamous relationships with a partner with chronic HCV infection, there was little evidence for sexual transmission of HCV (Clarke & Kulasegaram, 2006).

Currently the data are too scant to determine whether cosmetic procedures (e.g. tattooing, body piercing) or intranasal illicit drug use significantly contribute to the overall HCV transmission (Alter, 2002; Hwang *et al.*, 2006).

1.2.3 Persistence, latency, and natural history of infection

Persistence of HCV infection occurs in the majority of HCV-infected individuals. HCV infection is often asymptomatic. Indeed, acute HCV infection, whether symptomatic or not, resolves spontaneously only in 10–40% of cases (Poynard *et al.*, 2003; Afdhal, 2004). Persistent HCV infection is characterized by the persistence of elevated aminotransferase levels and HCV RNA in serum. Serological distinction of chronic carriers is difficult. Chronically HCV-infected patients are in general asymptomatic, and some report nonspecific symptoms such as fatigue or abdominal discomfort. Approximately 15–27% of chronically infected patients are estimated to develop cirrhosis. The time to progression to severe liver disease is highly variable. Factors that accelerate clinical progression include being of masculin gender, older at the age of infection, alcohol intake, and co-infection with HIV and/or HBV (Lauer & Walker, 2001; Perz *et al.*, 2006; Alter, 2007).

1.2.4 Vaccination and viral treatment

To date, an active or passive vaccination against HCV is not yet available. The main factor that hampers the development of an efficient vaccine is the considerable genetic heterogeneity of this positively-stranded RNA virus. However, better understanding of the natural immunity to HCV and the proof of vaccine efficacy in the chimpanzee challenge model allows some optimism about the development of an at least partly effective vaccine against this heterogeneous pathogen (Houghton & Abrignani, 2005).

In view of the fact that the natural course of chronic HCV infection is variable and that the currently established antiviral combination therapy with a pegylated interferon (PEG-IFN) and ribavirin (RBV) has numerous side-effects, the decision to treat or not to treat must be determined on an individual basis (Poynard *et al.*, 2003; Afdhal, 2004; Deutsch & Hadziyannis, 2008). The main goal of antiviral therapy is a sustained virological response, defined as undetectable HCV RNA in serum 24 weeks after the end of treatment, determined with the most sensitive PCR technique. Treatment, regimens, and responsiveness vary depending of the HCV genotypes.

In patients infected with genotype 1 or 4, HCV eradication rates range between 45–52%. In contrast, in patients infected with HCV genotype 2 or 3, antiviral therapy results in HCV eradication in 75–90% of cases. Currently, several novel antiviral agents are being evaluated in individual studies, e.g. NS3–4A protease inhibitors, RNA-dependent RNA polymerase inhibitors, and different immune therapies (Manns *et al.*, 2007).

2. Cancer in Humans

This section focuses on cohort and case–control studies published since the last *IARC Monograph* (IARC, 1994). Only those studies that used second- or third-generation assays to determine HCV antibody status are examined. If a measure of relative risk (RR) was not provided by the authors, the Working Group calculated a crude relative risk and 95% confidence intervals (CI). Only those studies in which a relative risk could be specifically estimated were included. Studies that focused specifically on the effect of the interaction of HCV and another factor (e.g. HBV) on the occurrence of hepatocellular carcinoma (HCC) are addressed in the relevant subsection.

2.1 Hepatocellular carcinoma

In the previous *IARC Monograph* (IARC, 1994), data on the relationship between infection with HCV, as indicated by the presence of antibodies to HCV (anti-HCV), were examined in three cohort studies and over 20 case–control studies. Seropositivity for HCV antibodies was measured by either first- or second-generation tests. An increased risk for HCC was apparent in all three cohort studies. Among the case–control studies, odds ratio (OR) estimates ranging from 1.3–134 were observed in 17 studies in which first-generation tests were used, and were statistically significant in 15 of the studies. In six case–control studies that used second-generation assays, the estimated odds ratios ranged from 1.1–52, and were significant in three studies. In the few case–control studies in which the analysis took into account possible confounding of the effects of HCV by other risk factors for HCC, such as smoking and alcohol consumption, the association with HCV was not materially altered.

2.1.1 Cohort studies

Table 2.1 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.1.pdf) provides a detailed summary of the cohort studies that investigated the association of HCV with the development of HCC, by geographic area. Cohort studies of patients with chronic liver disease/cirrhosis were excluded, due to the difficulties in interpreting the findings from such studies; this exclusion is analogous to that for case–control studies involving control groups comprising chronic liver disease patients.

Of the eight cohort studies considered, six were conducted in Asia and one each in the Americas and Australia. Of note, the cohort study conducted in Australia (Amin *et al.*, 2006) involved persons included in the New South Wales Notifiable Diseases Database. An association between HCV seropositivity and HCC was observed in each of the eight cohort studies, with relative risks ranging from 2.5–88. The effect estimate was statistically significant in all but one study (Yuan *et al.*, 1995). Potential confounding by risk factors for HCC, particularly infection with HBV, was not addressed in four of the studies (Yuan *et al.*, 1995; Boschi-Pinto *et al.*, 2000; Mori *et al.*, 2000; Guiltinan *et al.*, 2008). The wide range in the relative risks reported most likely reflects variations across the study populations in the underlying prevalence of HCV, as well as in the duration of HCV infection. Only the study by Mori *et al.* (2000) examined the effect of anti-HCV titre and reported a stronger association for a high anti-HCV titre (RR, 40.4) than for a low antibody titre (RR, 3.4).

2.1.2 Case–control studies

Details of the 18 case–control studies summarized can be found in Table 2.2 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.2.pdf). A few studies were excluded based on the following criteria: less

than 20 HCC cases were included in the study; the control group was comprised of patients with chronic liver disease; or, information related to the comparability of case and control subjects with respect to age and sex was not provided.

Most of the case–control studies were performed either in Europe ($n = 7$) or Asia ($n = 6$). The results from all 18 studies, both hospital-based and population-based, support a carcinogenic role of HCV in the development of HCC. The adjusted odds ratios for anti-HCV seropositivity ranged from 2.8–170; eight studies reported a more than 20-fold increased risk of HCC (Park *et al.*, 1995; Shin *et al.*, 1996; Tanaka *et al.*, 1996; Tsai *et al.*, 1996; De Vita *et al.*, 1998; Tagger *et al.*, 1999; Kuper *et al.*, 2000; Yuan *et al.*, 2004). Potential confounding by risk factors for HCC, particularly infection with HBV, was addressed in half of these studies (see Table 2.2 on-line). As with the cohort studies, the wide range in the observed odds ratios across the case–control studies is likely to be related to the underlying prevalence of HCV, and duration of infection. In those case–control studies in which the presence of anti-HCV and HCV RNA was determined, the observed association was stronger for positivity to both markers than for anti-HCV alone (Kew *et al.*, 1997; Tagger *et al.*, 1999; Gelatti *et al.*, 2005; Franceschi *et al.*, 2006a).

2.1.3 Meta-analyses

Two meta-analyses have examined the interactive effect of HBV and HCV infections on the occurrence of HCC (see the *Monograph* on HBV in this volume) based on case–control studies (including case–control studies nested within cohorts). In both analyses, estimates of the overall association between HCV and HCC were also provided. Donato *et al.* (1998) calculated a summary odds ratio for 21 studies, published between 1992–97, using second-generation anti-HCV or HCV RNA assays. The overall odds ratio for HCV was 8.2 (95%CI: 6.7–9.9). The estimate was higher for studies in areas where HBV infection is at low-to-intermediate endemicity and where HCV infection is predominant among HCC cases (Japan and Mediterranean countries; OR, 16.8; 95%CI: 11.9–24.1) than for studies in areas where HBV infection is highly endemic (subSaharan and southern Africa, Taiwan (China), China, Republic of Korea, Viet Nam; OR, 6.2; 95%CI: 4.9–7.8). The summary odds ratio was also slightly higher for studies with community controls (OR, 9.0; 95%CI: 7.0–11.6) than for those with hospital controls (OR, 6.8; 95%CI: 5.1–9.1). In a similar analysis of 32 case–control studies published in the Chinese literature, Shi *et al.* (2005) reported a summary odds ratio of 4.6 (95%CI: 3.6–5.9) for anti-HCV/HCV RNA positivity and HCC. The calculated odds ratio was somewhat larger in higher HCC incidence areas (OR, 5.3; 95%CI: 3.8–7.4) than in lower incidence areas (OR, 3.8; 95%CI: 2.8–5.2). There was little difference in the estimates based on studies with community controls or with hospital controls (OR, 4.7; 95%CI: 3.6–6.1 and OR, 4.4; 95%CI: 2.9–6.6, respectively).

2.1.4 HCV genotype

Table 2.3 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.3.pdf) summarizes the results from four case–control studies and one community-based cohort study in which the effect of HCV genotype on HCC was examined. Two case–control studies in Japan reported significant increased odds ratios for HCV genotype 1b, compared to other genotypes, among persons infected with HCV (Tanaka *et al.*, 1996, 1998a). A weaker crude association between Group 1 (1a, 1b) infection and HCC incidence was obtained from the nested case–control study of HCC conducted within the community-based Town C HCV Study (Suruki *et al.*, 2006). The Brescia HCC study examined the HCV genotype in the HCV RNA-positive HCC cases and non-cancer patient controls

(Tagger *et al.*, 1999), and reported a larger effect size for genotype 1b infection (OR, 34.2) than for genotype 2 infection (OR, 14.4), relative to anti-HCV seronegatives. A comparison of HCV genotypes 1b to 2 yielded an adjusted odds ratio of 2.9 (95%CI: 0.9–10) (Donato *et al.*, 1997). In contrast, an additional hospital-based study in Italy observed increased risks (27-fold) of HCC for both genotype 1 and genotype 2 infections, compared to anti-HCV seronegatives (Franceschi *et al.*, 2006a).

Seven cohort studies (Bruno *et al.*, 1997, 2007; Naoumov *et al.*, 1997; Niederau *et al.*, 1998; Tanaka *et al.*, 1998b; Ikeda *et al.*, 2002; Imazeki *et al.*, 2003a; Obika *et al.*, 2008) and two case–control studies (Hatzakis *et al.*, 1996; Silini *et al.*, 1996) have examined the association between HCV genotype and HCC among patients with HCV-related liver disease. However, uncertainty about HCV treatment in these patients as well as potential bias related to their identification and inclusion as study subjects make the interpretation of the findings from such studies difficult.

2.1.5 Cofactors modifying the risk of HCV-associated HCC

A brief summary of the findings from studies that examined potential modifiers of the effect of HCV on the development of HCC is provided below and in Table 2.4 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.4.pdf). Those studies that evaluated the interaction of the factor with a combined HCV and/or HBV infection status were not considered.

(a) Heavy alcohol consumption

Alcohol consumption has been classified by IARC as a human carcinogen, with a role in the etiology of liver cancer (IARC, 1988, 2010). A positive interaction, on the additive scale, between HCV and habitual alcohol drinking was observed in a community-based cohort study in Japan (Mori *et al.*, 2000); of note, only crude relative risks could be calculated for this study. Sun *et al.* (2003) also investigated the potential synergy of HCV infection with habitual alcohol drinking in their cohort study in Taiwan, China (Sun *et al.*, 2003; Wang *et al.*, 2003), and no interaction was observed in this low (20% prevalence) alcohol consumption population. The synergy index obtained suggested that the combined effect of HCV and habitual alcohol drinking was not more than the additive effect of either factor alone. A positive, more than additive, interaction between HCV and heavy alcohol consumption was reported in the hospital-based Brescia HCC case–control study in Italy (Donato *et al.*, 2002). However, because alcohol-induced cirrhosis may alter alcohol consumption before the development of HCC, the evaluation of alcohol in case–control studies can be problematic.

(b) Smoking

IARC has also identified tobacco smoking as a liver carcinogen (IARC, 2004). In the cohort studies in Japan (Mori *et al.*, 2000) and Taiwan, China (Sun *et al.*, 2003), a more than additive effect of anti-HCV seropositivity and cigarette smoking on HCC incidence was suggested; as noted in the alcohol subsection above, no adjustment for potential confounders was performed in the Japanese study.

(c) Diabetes mellitus

A meta-analysis of 26 studies (13 case–control studies and 13 cohort studies) published through February 2005 indicated that diabetes mellitus appears to be an independent risk factor for HCC (El-Serag *et al.*, 2006). However, no clear evidence with respect to a possible interaction between diabetes and HCV infection on the risk of HCC was found based on a Surveillance, Epidemiology and End Results(SEER)-Medicare database case–control study of HCC in the United States of America (Davila *et al.*, 2005),

and a community-based cohort study in Taiwan, China (Lai *et al.*, 2006).

(d) Betel quid chewing

Two studies in Taiwan, China, examined the interaction between HCV and habitual betel quid chewing (Sun *et al.*, 2003; Tsai *et al.*, 2001). The study by Sun *et al.* (2003) resulted in a synergy index of 4.2, suggestive of a greater than additive increased risk of HCC related to the combined effect of HCV infection and betel quid chewing than of either factor alone. The interaction appeared to be weaker in the study by Tsai *et al.* (2001), with a synergy index of 1.66; the reported odds ratios were not adjusted for potential confounders.

(e) Human T-lymphotropic virus type 1 infection

A more than additive effect of human T-lymphotropic virus type 1 (HTLV-1) co-infection on the association of HCV with liver cancer death was observed (RR, 21.9 for HCV-positive/HTLV-1-positive) in the Miyazaki Cohort Study of an HTLV-1 endemic population in Japan (Boschi-Pinto *et al.*, 2000).

(f) Radiation exposure

The Radiation Effects Research Foundation Life Span Study by Sharp *et al.* (2003) reported a greater than multiplicative interaction for the combined effect of HCV infection and radiation exposure in both the second (OR, 55.1) and third (OR, 28.7) tertiles.

(g) Schistosoma infection

A case–control study conducted at the National Cancer Institute in Egypt investigated the interaction between infection with HCV and infection with *Schistosoma mansoni* among the subjects negative for HBsAg (Hassan *et al.*, 2001); however, no meaningful interaction was observed.

(h) Helicobacter pylori infection

It has been hypothesized that *Helicobacter* species could play a role in the development of HCC (Pellicano *et al.*, 2008). Several cross-sectional studies have detected the presence of *Helicobacter* in HCV-infected HCC cases (Ponzetto *et al.*, 2000; Pellicano *et al.*, 2004; Rocha *et al.*, 2005).

2.1.6 Co-infection HCV/HBV

See the *Monograph* on HBV in this volume.

2.2 Cancers other than hepatocellular carcinoma

2.2.1 Biliary tract/gallbladder

(a) Cohort studies

The cohort study in Australia (Amin *et al.*, 2006), included in Table 2.1 (on-line), also investigated the effect of monoinfection with HCV on the incidence of gallbladder cancer; however, no increased rate of the malignancy was observed (standardized incidence ratio [SIR], 0.5; 95%CI: 0.1–2.0; based on 2 newly reported cases).

(b) Case–control studies

Table 2.5 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.5.pdf) summarizes the seven case–control studies that have examined the association of HCV with cholangiocarcinoma and biliary tract cancers, five of which were performed in Asian countries (Republic of Korea, Japan, and China).

In the Republic of Korea, a hospital-based study of 203 HCC cases (summarized in Table 2.2 on-line) also included 41 cases of cholangiocarcinoma, without distinction of site, and observed a positive association (OR, 3.9; 95%CI:0.9–17.1) of anti-HCV seropositivity with that malignancy (Shin *et al.*, 1996). Five case–control studies provided results with respect to the association

between anti-HCV seropositivity and intrahepatic cholangiocarcinoma. Statistically significant odds ratios were observed in three of those studies (Donato *et al.*, 2001; Yamamoto *et al.*, 2004; Shaib *et al.*, 2007), with a more than 5-fold increased risk of intrahepatic cholangiocarcinoma related to HCV. The largest study (Lee *et al.*, 2008), which included 622 cases in the Republic of Korea, did not find any association between anti-HCV positivity and intrahepatic cholangiocarcinoma (OR, 1.0; 95%CI: 0.5–1.9); of note, the reported odds ratio was not adjusted for any potential confounders. One study also included cases of extrahepatic cholangiocarcinoma (Shaib *et al.*, 2007), but the association between anti-HCV and this subtype was weaker, and not statistically significant. Not presented in Table 2.5 (on-line) is a case–control study based on the SEER cancer registry and Medicare claims data, which determined HCV infection status and case status using ICD-9 diagnostic codes (Welzel *et al.*, 2007). In that study as well, the effect was more pronounced for intrahepatic cholangiocarcinoma (adjusted OR, 4.4; 95%CI: 1.4–14.0; n = 535 cases) than for extrahepatic cholangiocarcinoma (adjusted OR, 1.5; 95%CI: 0.2–11.0; n = 549 cases).

Hsing *et al.* (2008) conducted a case–control study of incident biliary tract cancers in Shanghai. An age-adjusted increased risk associated with anti-HCV seropositivity was not observed for cancer of either the gallbladder, the extrahepatic bile ducts, or the ampulla of Vater.

2.2.2 Lymphoid malignancies

(a) Cohort studies

Table 2.6 (available at: http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.6.pdf) includes detailed descriptions of seven cohort studies that examined the effect of HCV infection on the occurrence of lymphoid malignancies, by the HIV status of the subjects. In the five studies of HIV-negative subjects, three found an approximately 2-fold excess risk of either non-Hodgkin lymphoma generally or B-cell non-Hodgkin lymphoma, specifically among individuals who were infected with HCV (Ohsawa *et al.*, 1999; Duberg *et al.*, 2005; Ulcickas Yood *et al.*, 2007). Due to the small number of cases that occurred in all three cohorts, the association was statistically significant only in the Swedish study of B-cell non-Hodgkin lymphoma (Duberg *et al.*, 2005). The Swedish study also observed a similar, but statistically non-significant, effect of HCV infection on the development of the chronic lymphocytic leukaemia subtype as well as multiple myeloma. A nested case–control study within a cohort of parents and offspring in the USA did not detect HCV in any of the B-cell non-Hodgkin lymphoma, multiple myeloma, or Hodgkin disease cases or the matched controls (Rabkin *et al.*, 2002). A cohort study in Australia also reported no association of HCV with non-Hodgkin lymphoma, nor with subtypes such as follicular lymphoma, diffuse non-Hodgkin lymphoma, and T-cell non-Hodgkin lymphoma (Amin *et al.*, 2006); in addition, an increased risk was not found for either multiple myeloma or Hodgkin disease. Not included in Table 2.6 is a cohort study from the USA, which used the Department of Veterans Affairs administrative patient databases with HCV status, and the occurrence of lymphoid malignancies based on ICD-9 CM diagnostic codes (Giordano *et al.*, 2007). The adjusted relative risks were 1.28 (95%CI: 1.12–1.45) for non-Hodgkin lymphoma (n = 1359 cases), 0.89 (95%CI: 0.68–1.15) for chronic lymphocytic leukaemia (n = 412 cases), 0.95 (95%CI: 0.76–1.19) for multiple myeloma (n = 526 cases), and 0.97 (95%CI: 0.74–1.27) for Hodgkin disease (n = 360 cases).

For both cohort studies of HIV-positive subjects, a null association was observed between anti-HCV seropositivity and non-Hodgkin lymphoma (Waters *et al.*, 2005; Franceschi *et al.*, 2006b).

(b) Case–control studies

This section summarizes the case–control studies included in the meta-analyses by Matsuo *et al.* (2004) and Dal Maso & Franceschi (2006). Other case–control studies were considered if: at least 20 cases of lymphoma were investigated in the study; the control group was not comprised of patients with other haematological malignancies and/or lymphoproliferative diseases; and, information related to the comparability of case and control subjects with respect to age and sex was provided.

Results related to a particular non-Hodgkin lymphoma subtype are included when data were provided for ≥ 20 cases. In addition to B-cell non-Hodgkin lymphoma, the subtypes evaluated were T-cell non-Hodgkin lymphoma, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, marginal zone lymphoma, and chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL).

A large number of case–control studies have examined the association between HCV infection and non-Hodgkin lymphoma. The detailed description of these studies and their findings, by geographic area, are provided in Table 2.7 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.7.pdf). Most studies were conducted in Europe (n = 20), followed by the Americas (n = 8), and Asia (n = 7); one study each was performed in Africa and Australia. B-cell non-Hodgkin lymphoma specifically and/or non-Hodgkin lymphoma generally was the primary malignancy of interest in all 37 case–control studies. Approximately 80% of the studies reported at least a 2-fold increased risk of non-Hodgkin lymphoma or a B-cell non-Hodgkin lymphoma with HCV seropositivity. The association was evident in case–control studies conducted across all geographic areas (except for the study in Australia), and using different sources of control groups (i.e. hospital-based, population-based, or blood donors). Odds ratios greater than 5.0 were observed in 13 studies: nine studies were from Europe (Ferri *et al.*, 1994; Mazzaro *et al.*, 1996; Musto *et al.*, 1996; De Rosa *et al.*, 1997; De Vita *et al.*, 1998; Paydas *et al.*, 1999; Vallisa *et al.*, 1999; Zucca *et al.*, 2000; Yenice *et al.*, 2003), and four studies were from elsewhere (Zuckerman *et al.*, 1997; Harakati *et al.*, 2000; Kuniyoshi etal., 2001; Chindamo *et al.*, 2002). An additional 16 case–control studies reported a 2–4-fold excess risk of either non-Hodgkin lymphoma or B-cell non-Hodgkin lymphoma associated with HCV infection; the effect was statistically significant in more than half (Silvestri *et al.*, 1996; Mizorogi *et al.*, 2000; Montella *et al.*, 2001b; Imai *et al.*, 2002; Mele *et al.*, 2003; Cowgill *et al.*, 2004; Engels *et al.*, 2004; Iwata *et al.*, 2004; Talamini *et al.*, 2004; Schöllkopf *et al.*, 2008; Spinelli *et al.*, 2008). The remaining eight case–control studies with odds ratios less than 2 did not demonstrate a statistically significant effect of HCV on the occurrence of non-Hodgkin lymphoma or B-cell non-Hodgkin lymphoma (Kaya *et al.*, 2002; Avilés *et al.*, 2003; Morgensztern *et al.*, 2004; de Sanjosé *et al.*, 2004; Sève *et al.*, 2004; Vajdic *et al.*, 2006; Sonmez *et al.*, 2007; Park *et al.*, 2008). Not included in Table 2.7 is the SMAHRT (SEER-Medicare Assessment of Hepatopoietic Malignancy Risk Traits) study, a case–control study of haematopoietic malignancies based on the use of the SEER-Medicare data in the USA (Anderson *et al.*, 2008). Cancer diagnosis as well as HCV status was obtained from the ICD codes included in the two databases. The adjusted odds ratio for the association of HCV with non-Hodgkin lymphoma overall (n = 33940 cases) was 1.35 (95%CI: 1.1–1.7).

The findings of two pooled analyses are also included in Table 2.7. One pooled study reported an overall odds ratio of 1.5 (95%CI: 0.95–2.2) for B-cell lymphoma, based on 1465 cases from five European countries (Nieters *et al.*, 2006). The other pooled study found a similar association (OR, 1.8; 95%CI: 1.4–2.3) for all non-Hodgkin

lymphoma, using 4784 cases from seven studies involving centres in the USA, Canada, Australia, and Europe (de Sanjosé *et al.*, 2008). The latter analysis included the results from five previously published studies (Engels *et al.*, 2004; Morton *et al.*, 2004; Talamini *et al.*, 2004; Nieters *et al.*, 2006; Vajdic *et al.*, 2006).

In two case–control studies of non-Hodgkin lymphoma, a slightly stronger association was found for low-grade B-cell non-Hodgkin lymphoma, in contrast to intermediate/high-grade disease (Engels *et al.*, 2004; Talamini *et al.*, 2004). Based on an *a priori* hypothesis that HCV would be related to non-Hodgkin lymphoma at the potential target organ of infection, an early study in Italy examined the effect of HCV on liver/salivary gland non-Hodgkin lymphoma, and reported a marked elevation in risk (OR, 51.5) (De Vita *et al.*, 1998).

With respect to other subtypes of non-Hodgkin lymphoma, an effect of HCV infection has less consistently been observed. Moreover, when an association is suggested, the effect estimate usually is highly unstable, due to the small number of cases involved. From the results of the studies included in Table 2.7 (on-line), an association with HCV seems more evident for the DLBCL and CLL/SLL subtypes. For DLBCL, results were available from 14 case–control studies; 13 studies observed an association consistent with an effect of HCV seropositivity on DLBCL, which was significant for about half (Silvestri *et al.*, 1996; Vallisa *et al.*, 1999; Zucca *et al.*, 2000; Chindamo *et al.*, 2002; Mele *et al.*, 2003; Talamini *et al.*, 2004; Spinelli *et al.*, 2008). Eight of the 12 studies that examined CLL/SLL reported an odds ratio of at least 2, with the association being statistically significant in three case–control studies (Musto *et al.*, 1996; De Rosa *et al.*, 1997; Paydas *et al.*, 1999). For the fewer number of studies (n = 10) that included separate odds ratios for follicular lymphoma and marginal zone lymphoma, slightly more than half reported an odds ratio greater than 2 for HCV seropositivity (Silvestri *et al.*, 1996; Vallisa *et al.*, 1999; Zucca *et al.*, 2000; Mele *et al.*, 2003; Engels *et al.*, 2004; Spinelli *et al.*, 2008). In contrast, the findings from almost all studies did not support a notable effect of HCV on T-cell non-Hodgkin lymphoma. The SMAHRT study reported the following odds ratios for non-Hodgkin lymphoma subtypes: 1.5 (95%CI: 1.05–2.2) for DLBCL (n = 10144 cases); 2.2 (95%CI: 1.2–3.95) for marginal zone lymphoma (n = 1908 cases); 1.9 (95%CI: 1.2–3.0) for follicular lymphoma (n = 4491 cases); 1.1 (95%CI: 0.70–1.7) for chronic lymphocytic lymphoma (n = 10170 cases); and 0.42 (95%CI: 0.10–1.7) for T-cell non-Hodgkin lymphoma (n = 1870 cases) (Anderson *et al.*, 2008). In a pooled analysis (de Sanjosé *et al.*, 2008), a statistically significant association was found for DLBCL (OR, 2.2; 95%CI: 1.7–3.0) and marginal zone lymphoma (OR, 2.5; 95%CI: 1.4–4.2). The other pooled study (Nieters *et al.*, 2006) also reported a significant association for DLBCL (OR, 2.2; 95%CI: 1.2–3.9), but no association was reported for marginal zone lymphoma. The effect of HCV was weaker and not statistically significant for CLL/SLL and not apparent for follicular lymphoma or T-cell lymphoma in both pooled studies.

Of eight case–control studies that examined multiple myeloma (Table 2.7 on-line), four studies observed a statistically significant association between HCV seropositivity and the malignancy (Musto *et al.*, 1996; De Rosa *et al.*, 1997; Paydas *et al.*, 1999; Montella *et al.*, 2001a, b). The pooled analysis by the EPILYMPH group (Nieters *et al.*, 2006) did not report a notable association for multiple myeloma (OR, 1.4; 95%CI: 0.61–3.2).

More than ten of the case–control studies included in Table 2.7 (on-line) conducted separate analyses of the effect of HCV on the occurrence of Hodgkin disease. An increased risk was observed in eight case–control studies, although it was significant only in one study (Paydas *et al.*, 1999). The US record-based study by Anderson *et al.* (2008) found an odds ratio of 1.2 (95%CI: 0.38–3.7) based on 1155 Hodgkin disease cases.

In the pooled analysis by Nieters *et al.* (2006), no effect of HCV on Hodgkin disease was found (OR, 0.97; 95%CI: 0.27–3.5).

(c) Meta-analyses

Several meta-analyses of the relationship between HCV and lymphoma have been published (Gisbert *et al.*, 2003; Matsuo *et al.*, 2004; Negri *et al.*, 2004; Dal Maso & Franceschi, 2006). Matsuo *et al.* (2004) performed a meta-analysis of 23 case–control studies (including nested case–control studies) published between January 1990 and August 2003. Studies were included if an odds ratio or a relative risk was calculated "by comparing the HCV-positive category to the negative category", and non-cancer controls and a second- or third-generation HCV antibody test were used. A summary odds ratio of 5.7 (95%CI: 4.1–8.0) for non-Hodgkin lymphoma was obtained; the association was somewhat stronger for B-cell non-Hodgkin lymphoma (OR, 5.0; 95%CI: 3.6–7.1) than for T-cell non-Hodgkin lymphoma (OR, 2.5; 95%CI: 1.4–4.6). The variation was related to the use of blood donor controls (OR: 8.4 versus OR: 4.65 for non-blood donor controls), and to the year of publication (i.e. lower OR for the more recent publications). [The Working Group noted that the relative risks obtained in this meta-analysis might be inflated by underadjustment, particularly by age group.]

In their meta-analysis, Dal Maso & Franceschi (2006) examined 15 case–control studies and three cohort studies published up to July 2006. Studies were eligible for inclusion based on the following criteria: if the control group did not include patients with other lymphoproliferative diseases, if cases and controls were comparable with respect to age and sex or age and sex were taken into account in the analysis, if second- or third-generation anti-HCV assays were used, and if HIV-positive subjects were excluded. Only case–control studies with ≥ 100 cases were included in the overall analyses of non-Hodgkin lymphoma; findings regarding specific non-Hodgkin lymphoma subtypes and other lymphoid malignancies were shown when there were ≥ 20 cases for that subtype. The sex- and age-adjusted meta-relative risk for all non-Hodgkin lymphoma was 2.5 (95%CI: 2.1–3.0), and was similar for case–control (RR, 2.5; 95%CI: 2.1–3.1) and cohort (RR, 2.0; 95%CI: 1.8–2.2) studies. The effect of HCV on non-Hodgkin lymphoma was estimated to be higher in those studies with a higher HCV prevalence (≥ 5%) among control subjects (RR, 3.0; 95%CI: 2.4–3.75) than those studies with a lower prevalence (RR, 1.9; 95%CI: 1.8–2.05). The results were relatively consistent by geographic area (RR, 2.7 in southern Europe; RR, 2.6 in the USA; RR, 2.1 in the Republic of Korea/Japan; RR, 2.3 in other areas) and year of publication (RR, 2.9 before 2003; RR, 2.2 during 2003 and after). However, the association appeared to be somewhat weaker in studies using population-based control subjects (RR, 1.9) than in those using hospital-based controls (RR, 2.7). With respect to non-Hodgkin lymphoma subtypes, the relative risks obtained were: 2.65 (95%CI: 1.9–3.7) for DLBCL; 2.7 (95%CI: 2.2–3.4) for follicular lymphoma; 3.4 (95%CI: 2.4–4.9) for marginal zone; 1.65 (95%CI: 1.35–2.0) for CLL/SLL; and 1.5 (95%CI: 1.13–2.05) for T-cell non-Hodgkin lymphoma. In addition, a summary relative risk was estimated for multiple myeloma (RR, 1.6; 95%CI: 0.69–3.6) as well as for Hodgkin disease (RR, 1.5; 95%CI: 1.0–2.1). Of note, the meta-analysis did not include the results from two recently conducted, large case–control studies of lymphoid malignancies: the nationwide study of the Danish and Swedish populations by Schöllkopf *et al.* (2008), and the pooled InterLymph study by de Sanjosé *et al.* (2008).

(d) Treatment of HCV infection in patients with lymphoma

In a striking finding, Hermine *et al.* (2002) reported remission in seven of nine patients with splenic lymphoma with villous lymphocytes who

were infected with HCV after they were treated with interferon α; two more patients without a substantial antiviral response experienced remission after the addition of ribavirin to interferon α. None of six HCV-negative patients with splenic lymphoma with villous lymphocytes responded to interferon treatment. A systematic review by Gisbert *et al.* (2005) reported on the findings of 16 case reports and case series in which a total of 65 HCV-infected patients with lymphoproliferative disorders were treated with interferon α (with and without ribavirin). Of the 65 patients in those studies, complete remission of the disorder was achieved in 75% (95%CI: 64–84) of the cases. [The Working Group noted that one case series contributed almost a third of the patients analysed (n = 20); however, the reference was to an abstract, for which a full paper has not been published.]

2.2.3 Other cancers

(a) Leukaemias

In the large cohort study of US veterans who were infected with HCV, a diagnosis of HCV was not related to an increased rate of acute lymphocytic leukaemia (RR, 0.75), chronic myeloid leukaemia (RR, 0.84), or acute non-lymphocytic leukaemia (RR, 1.04) (Giordano *et al.*, 2007).

Table 2.8 (available at http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.8.pdf) summarizes the four case–control studies that investigated the effect of HCV infection on the development of leukaemia. No study reported statistically significant associations between anti-HCV seropositivity and acute myeloid leukaemia, acute lymphocytic leukaemia, or chronic myeloid leukaemia. However, Bianco *et al.* (2004) suggested that HCV infection might be associated with both acute myeloid leukaemia and chronic myeloid leukaemia in the Italian study. In Japan, Murashige *et al.* (2005) reported no association of anti-HCV seropositivity with the risk of myeloid malignancy.

(b) Cancer of the thyroid

The three cohort studies in Sweden (Duberg *et al.*, 2005), Australia (Amin *et al.*, 2006), and the USA (Giordano *et al.*, 2007) examined the incidence of thyroid cancers among people who were infected with HCV. A non-significant increased risk was found based on five thyroid cancer cases in the Swedish cohort (SIR, 1.55; 95%CI: 0.50–3.6); whereas a significant decreased risk was reported based on nine cases in the Australian cohort (SIR, 0.3; 95%CI: 0.2–0.7), and 46 cases in the US cohort (HR, 0.72; 95%CI: 0.52–0.99). A case–control study of a group of cancers in Italy (Montella *et al.*, 2001a) observed a significant association between HCV infection and this malignancy (OR, 2.8; 95%CI: 1.2–6.3).

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

The sharp increase of HCC cases over the last several decades in industrialized countries (western Europe, North America, and Japan) has been attributed to an expansion of chronic HCV infections, and is frequently linked with liver cirrhosis. Most cohort studies and clinical experience have shown that liver fibrosis resulting from long-lasting chronic inflammation is likely to be an important predisposing factor of HCC development. The same clinical observations also suggest that ongoing liver regeneration resulting from chronic immune mediated hepatocyte

death is a likely factor contributing to the development of HCC (Liang & Heller, 2004). This aspect has been developed in the *Monograph* on HBV in this volume.

But the mechanisms underlying the progression of HCV infection to liver cancer, which often takes many decades, remain ill-defined. Transcriptomics and proteomics have helped to identify many genetic and epigenetic alterations associated with HCC clusters. However, the identified changes to gene expression patterns are very heterogeneous in tumour cells, raising the question as to whether yet unidentified, specific changes at early, preneoplastic stages trigger the transformation process, and what cell type could be at the origin of HCC (Sell & Leffert, 2008). HCV has an exclusively cytoplasmic life cycle (Lindenbach *et al.*, 2007; Moradpour *et al.*, 2007), and therefore, HCV replication and potentially pro-oncogenic events are restricted to the cytoplasm. While HCV infection leads to chronic inflammation, steatosis, fibrosis and oxidative chromosomal DNA damage, several HCV proteins have been shown to have direct oncogenic effects, and to upregulate mitogenic processes (Koike, 2007; McGivern & Lemon, 2009). The accumulation of DNA damage within a setting of restricted cell-cycle checkpoint controls is thought to compromise gene and chromosome stability, and to form the genomic basis for the malignant phenotype.

One of the major challenges with the study of the carcinogenic role of HCV is the difficulty to localize the HCV genome and viral proteins in both the liver of infected patients and liver tumours.

4.1 Biochemical properties of HCV proteins

See Table 4.1.

4.1.1 The structural proteins

(a) Core protein

The core protein is the most conserved among the structural proteins. It is highly basic, rich in proline, and multimerizes. Interaction of the mature core with the 5′ and 3′ NCRs can, respectively, inhibit IRES function, and mediate genome dimerization (Shimoike *et al.*, 1999; Cristofari *et al.*, 2004). The core protein is targeted to the cytoplasmic surface of the endoplasmic reticulum and lipid droplets (Moradpour *et al.*, 1996; Barba *et al.*, 1997). Its interaction with lipid droplets may be related to the increased incidence of liver steatosis in patients with HCV, and in certain transgenic mice that overexpress HCV core (Moriya *et al.*, 1997a; Barbaro *et al.*, 1999). It has also been shown that core can localize to mitochondrial outer membranes (Schwer *et al.*, 2004).

(b) HCV glycoproteins

The HCV glycoproteins, E1 (30 kDa) and E2 (70 kDa), are type I transmembrane glycoproteins that associate into non-covalently attached heterodimers, and mediate HCV cell entry and membrane fusion (Bartosch & Cosset, 2006; Lavillette *et al.*, 2006; Lindenbach *et al.*, 2007).

4.1.2 The non-structural proteins

(a) p7

p7 is a small 7 kDa hydrophobic protein predicted to span the membrane twice, with endoplasmic-reticulum-lumenal *N*- and *C*-terminals and a short, positively charged cytoplasmic loop. The localization of p7 is not yet clear, but overexpression of an epitope-tagged p7 has been localized to the endoplasmic reticulum and mitochondria (Griffin *et al.*, 2005). The p7 protein is essential for virus assembly and release *in vitro* (Steinmann *et al.*, 2007), and infectivity *in vivo* (Sakai *et al.*, 2003).

Table 4.1 HCV proteins, their role in the viral life cycle, and putative role in hepatocyte transformation

HCV proteins	Role in viral life cycle	Potential role in cellular transformation (examples)
Core protein	Nucleocapsid assembly	Insulin resistance/steatosis Interference (direct or indirect) with p53, p73, pRb Interference with host cell signalling (NF-κB, Wnt/β-catenin pathway) Interference with TGF-β signalling Transcriptional activation of cellular genes Apoptosis
E1/E2 glycoprotein	Virus morphogenesis Cell entry	Interference with the interferon-inducible protein kinase (PKR) activity
p7	Virus assembly, export and infectivity	
NS2	Polyprotein processing and Viral assembly	Inhibition of apoptosis
NS3 *N*-terminal domain	Serine protease activity	Interference with hepatocyte innate response
NS3 *C*-terminal domain	Helicase activity HCV genome replication	
NS4A	Co-factor of NS4B and NS5A	Induction of ER stress
NS4B	Formation of membranous web structures	
NS5A	Part of the replication complex	Inhibition of the interferon-inducible PKR Oxydative stress Activation of cell signalling pathways (STAT-3, NF-κB etc) Accumulation of β-catenin by indirect mechanism
NS5B	RNA-dependent RNA polymerase	

ER, endoplasmic reticulum; PKR, double-stranded RNA-activated protein kinase
Compiled by the Working Group

(b) NS2

NS2 is a 23 kDa membrane-spanning protein with a *C*-terminal cysteine protease activity that cleaves the NS2/3 junction (Grakoui *et al.*, 1993; Lorenz *et al.*, 2006). NS2 is furthermore required for viral assembly. Its *N*-terminal domain may interact with the structural proteins and p7, whereas downstream sequences may interact with other NS proteins (Lindenbach *et al.*, 2007; Jirasko *et al.*, 2008).

(c) NS3

HCV NS3 is a 70 kDa multifunctional protein, containing an *N*-terminal serine protease domain and a *C*-terminal RNA helicase/NTPase domain that unwinds double-stranded nucleic acids (Lindenbach *et al.*, 2007). Although the precise role of the NS3 helicase is not yet known, helicase activity has been shown to be essential for HCV RNA replication and viral infectivity (Lam & Frick, 2006).

(d) NS4A

NS4A is an 8 kDa protein with multiple functions in the virus life cycle. It is a cofactor that assists in the correct folding of the serine protease, and facilitates recognition of RNA substrates by the NS3 protease/helicase (Pang *et al.*, 2002). NS4A can physically interact with NS4B and NS5A and uncleaved NS4B-5A to promote NS5A hyperphosphorylation (Lindenbach *et al.*, 2007; and references therein).

(e) NS4B

NS4B is a 27 kDa integral membrane protein containing four central transmembrane domains with yet unclear topology. Its expression is sufficient to induce the formation of 'membraneous web' structures that contain the membrane-bound replication complex (Egger *et al.*, 2002; Gosert *et al.*, 2003), and it encodes a GTPase activity that seems to be critical for RNA replication (Einav *et al.*, 2004).

(f) NS5A

NS5A is a 58 kDa phosphoprotein with an important yet unclear role in RNA replication (Shimakami *et al.*, 2004). It localizes to active replication complexes (Gosert *et al.*, 2003; Moradpour *et al.*, 2004), interacts with NS5B and inhibits its RNA polymerase activity (Shirota *et al.*, 2002; Dimitrova *et al.*, 2003).

(g) NS5B

NS5B is a 68 kDa endoplasmic-reticulum-membrane-associated protein with RNA-dependent polymerase activity. Mutations that interfere with its membrane association destroy RNA replication. Intramolecular interactions as well as oligomerization of NS5B stimulate RNA synthesis, and the NS3 helicase enhances primed RNA synthesis activity in contrast to NS4B and NS5A, which inhibit RNA synthesis (Lindenbach *et al.*, 2007; and references therein). NS5B has been and remains a major target for the development of HCV-specific drugs; at the time of writing, drug research and development is focusing on cellular cofactors of NS5B, the cyclophilins. The function of NS5B has been shown to be upregulated by cyclophilin B, which in turn is regulated, and thus sensitive to the immunosuppressant ciclosporin A. Compounds belonging to this family are currently investigated for their antiviral efficacy (Watashi *et al.*, 2005; Watashi & Shimotohno, 2007).

4.2 Biological properties of HCV proteins

See Table 4.1.

[The Working Group noted that besides the complex interactions among themselves, the viral proteins interact with a significant number of host factors and signalling pathways that may contribute to the pathological consequences of HCV infection. These interactions interfere with innate immunity and thus contribute to persistence of infection and inflammation; but they have also been described to modulate transcription, translation and post-translational events, to alter cell signalling, apoptosis, membrane physiology and trafficking. Furthermore, they can induce oxidative stress, genomic instability and possibly cellular transformation.

Many studies of the potential role of viral proteins in hepatocyte transformation have been performed in experimental models that are based on the overexpression of viral proteins after transient transfection of already transformed hepatocytes (such as HepG2 or Huh7 cells). These studies show the interaction of viral proteins with cellular partners that may be involved in cellular transformation. However, because the expression of these viral proteins has been difficult to demonstrate in liver tumours, a link between these *in vitro* observations and their *in vivo* relevance in infected humans still needs to be established. Because of the lack of relevant models for mechanistic studies of HCV-induced HCC, the results of the major molecular studies have been described below to provide an overview of the current hypotheses.]

Of the HCV gene products core, NS3, NS4B and NS5A have all been shown to exhibit transformation potential when transiently or stably expressed in tissue culture, or in the context of transgenic mice carrying the single viral proteins or an HCV polyprotein (Sakamuro *et al.*, 1995; Ray *et al.*, 1996; Gale *et al.*, 1999; Park *et al.*, 2000).

However, many of the data below need to be substantiated in the context of a viral infection.

4.2.1 The structural proteins

(a) Core protein

Core has been implied in changes of host cell signalling, transcriptional activation, apoptosis, lipid metabolism, and transformation. Among an impressive list of interactions with cellular factors, core has been shown to physically and functionally interact with p53 (Ray *et al.*, 1997; Lu *et al.*, 1999), and p73 (Alisi *et al.*, 2003), and to decrease the expression of pRb (Cho *et al.*, 2001) tumour-suppressor proteins. For instance, it was shown that HCV core co-immunoprecipitates with p73 in HepG2 and SAOS-2 cells. This interaction results in the nuclear translocation of HCV core protein. In addition, the interaction with HCV core protein prevents p73-α-, but not p73-β-dependent cell growth arrest in a p53-dependent manner. The results suggested that HCV core protein may directly influence the various p73 functions, thus playing a role in HCV pathogenesis (Alisi *et al.*, 2003).

Core also modulates the expression of the cyclin-dependent kinase (CDK) inhibitor p21/Waf in a p53-independent manner (Kwun & Jang, 2003). p21, a well known transcriptional target of p53, blocks activities of cyclin/CDK complexes involved in cell-cycle control and tumour formation.

Core induces activation of the Raf1/mitogen-activated protein kinase (MAPK) pathway (Aoki *et al.*, 2000; Hayashi *et al.*, 2000), relieves cells from serum starvation and growth arrest, and favours cell proliferation.

Conflicting reports have shown both activation (Ray *et al.*, 2002) and repression (Joo *et al.*, 2005) of the NF-κB pathways by HCV core.

HCV core has been shown to activate the Wnt/β-catenin pathway, which is implicated in cell proliferation, DNA synthesis, and cell-cycle progression (Fukutomi *et al.*, 2005).

Furthermore, core variants isolated from liver tumours, but not those isolated from adjacent non-tumourous liver, have been shown to interact with Smad3 and inhibit the TGF-β pathway (Pavio *et al.*, 2005). TGF-β-signalling not only controls cell proliferation, differentiation and apoptosis but also stimulates liver regeneration and fibrogenesis through its actions on the extracellular matrix. TGF-β levels are frequently increased in chronic HCV patients and correlate with the degree of fibrosis (Nelson *et al.*, 1997; Marcellin *et al.*, 2002).

Finally, HCV core protein associates with cellular membranes (Barba *et al.*, 1997; Moriya *et al.*, 1997a) and lipid vesicles (Moriya *et al.*, 1997a), binds to apolipoprotein II, and reduces microsomal triglyceride transfer protein (MTP) activity (Perlemuter *et al.*, 2002), leading to defects in the assembly and secretion of vLDL and steatosis, which in turn induces oxidative stress. The *in vivo* relevance of this interaction is supported by the development of steatosis (Moriya *et al.*, 1997b; Perlemuter *et al.*, 2002) and liver cancer (Moriya *et al.*, 2001; Lerat *et al.*, 2002) in transgenic mice expressing HCV core.

(b) E2

Overexpression of E2 inhibits eIF2α phosphorylation by the dsRNA-activated protein kinase (PKR) or the endoplasmic-reticulum-stress signalling kinase PERK. E2 also physically interacts with PKR; the E2/PKR interaction may account for the intrinsic interferon's resistance of HCV genotypes 1a and 1b (Taylor *et al.*, 1999; Pavio *et al.*, 2003).

4.2.2 The non-structural proteins

(a) NS4A, NS4B or NS4A-4B

Overexpression of NS4A, NS4B, or NS4A-4B has been reported to induce an endoplasmic-reticulum- stress-mediated unfolded protein response, reduce endoplasmic-reticulum-to-Golgi traffic, inhibit protein synthesis, and to

cause cytopathic effects (Lindenbach *et al.*, 2007; and references therein).

(b) NS2

NS2 interacts with the cellular proapoptotic molecule CIDE-B and inhibits CIDE-B-induced apoptosis (Erdtmann *et al.*, 2003). NS2 has also been shown to downregulate the transcription of several cellular and viral promoters in gene-reporter assays (Dumoulin *et al.*, 2003).

(c) NS3-4A

NS3-4A serine protease has been reported to block the activation of the transcription factors IRF-3 and NF-κB, and to antagonize innate antiviral defenses by interfering with the cytosolic RNA helicases, RIG-1- and MDA5-, and TLR3-mediated signal transduction (Lindenbach *et al.*, 2007).

(d) NS5A

NS5A has multiple functions. It has been shown to interact with the geranylgeranylated cellular protein FBL2 (Wang *et al.*, 2005), an F-box-motif-containing protein that is likely to be involved in targeting cellular proteins of yet unknown identity for ubiquitylation and degradation.

Several studies suggest that NS5A is also involved in the resistance to interferon treatment (Lindenbach *et al.*, 2007; and references therein), and one possible mechanism may be its ability to induce expression of the type I interferon antagonist IL-8 (Polyak *et al.*, 2001). In addition, NS5A contains an 'interferon sensitivity determining region' (ISDR) that mediates inhibition of PKR, an activator of innate immunity; accumulation of mutations in this region is thought to correlate with treatment efficacy (Enomoto *et al.*, 1995, 1996).

Overexpression of NS5A has been reported to induce several effects in cells, including oxidative stress; activation of signalling pathways, including STAT-3, PI3K, and NF-κB (Gong *et al.*, 2001; He *et al.*, 2002; Street *et al.*, 2004); and degradation of pRB (Munakata *et al.*, 2005).

Other reported NS5A interaction partners include apolipoprotein A1, the major protein found on High Density Lipoprotein (HDL); the tumour suppressor, p53; Grb-2, an adaptor protein involved in mitogen signalling; SRCAP, an adenosine triphosphatase (ATPase) that activates cellular transcription; karyopherin β3, a protein involved in nuclear trafficking; Cdk1/2, cyclin-dependent and Fyn, Hck, Lck, and Lyn, Src-family kinases (Lindenbach *et al.*, 2007; and references therein).

More recently, it has been reported that NS5A-expression in the context of HCV polyprotein results in the inhibition of the transcription factor Forkhead as well as in the phosphorylation and inactivation of the GSK-3, leading to the accumulation of β-catenin and to the stimulation of β-catenin-dependent transcription (Street *et al.*, 2005).

[The Working Group noted that, so far, the biological functions of the HCV proteins have been investigated *in vitro* or *in vivo* using transgenic mice constitutively expressing HCV proteins alone, in combination, or the entire polyprotein. Whether the results generated by these experimental approaches reflect the pathological consequences of an HCV infection *in vivo* remains to be addressed, and this issue will only be resolved with the establishment of immunocompetent mouse models or other more physiological cellular models that permit chronic and productive HCV replication.]

4.3 Experimental evidence for a role of HCV in malignant conversion

4.3.1 Role of HCV chronic infection in HCC development

Successful clearance of chronic HCV infection has been shown to reduce the overall liver-related mortality and the incidence of HCC providing further evidence for a causal role of HCV in this cancer (Kasahara *et al.*, 1998; Serfaty *et al.*, 1998; Imazeki *et al.*, 2003b).

Although chronic HCV infection is one major risk factor for HCC, the mechanisms by which HCV induces HCC remain uncertain (Levrero, 2006; McGivern & Lemon, 2009). Chronic endoplasmic reticulum stress and inflammatory responses and the associated oxidative stress and altered intracellular redox state lead to the accumulation of genomic damage. These are likely to contribute to and predispose infected cells to hepatocarcinogenesis, possibly via changes in MAPK signalling, that regulates both cell metabolism and growth (Tardif *et al.*, 2002; Waris *et al.*, 2007). Markers of intracellular oxidative stress have indeed been reported to be increased in chronic HCV patients (Shimoda *et al.*, 1994; Sumida *et al.*, 2000) as well as in HCV core transgenic mice (Moriya *et al.*, 1998; Moriya *et al.*, 2001). However, in addition, direct interactions of the various HCV proteins with host factors correlate with changes in cellular signalling cascades that are involved in the regulation of cell metabolism and division. The expression of some HCV proteins seem to be sufficient to induce hepatocarcinogenesis, at least in some specific transgenic mice lineages such as transgenic C57BL/6 mice (Lerat *et al.*, 2002); liver tumour development was shown to be associated with HCV-induced liver steatosis (Lerat *et al.*, 2002; Moriya *et al.*, 1998).

Overall, because of the lack of an experimental model that replicates the viral life cycle and natural history of the disease, the current view is that synergistic effects between the consequences of chronic inflammation and direct virus–host cell interactions are most likely. Such synergistic effects would also explain the long 'multistep' transformation process in human HCC, which is consistent with the long time lag with which cirrhosis and HCC manifest themselves upon chronic infection, and would explain the wide variety of genetic defects observed in individual HCCs (Thorgeirsson & Grisham, 2002; Levrero, 2006; McGivern & Lemon, 2009).

Prospective and retrospective cohort studies of patients with chronic HCV infection have demonstrated a role for the long duration of the disease in HCC development, and the link between HCC development and liver cirrhosis. These studies showed the sequential occurrence of advanced liver fibrosis followed by the development of HCC. The incidence of HCC development was estimated to be between 3–5%/year in cirrhotic patients (Tsukuma *et al.*, 1993; Tong *et al.*, 1995; Fattovich *et al.*, 1997).

4.3.2 Role of HCV-induced steatosis, insulin resistance, and oxidative stress in HCC development

Pro-carcinogenic cofactors in chronic HCV infection are steatosis, oxidative stress and insulin resistance, suggesting many parallels with non-alcoholic fatty liver disease (NAFLD). In NAFLD, chronic excess of nutrients causes endoplasmic reticulum stress, and leads to an increase of hepatic fat (steatosis) and insulin resistance; a complex interplay between these factors can lead to chronic liver inflammation, apoptosis and fibrogenesis, which are thought to form the prelude to liver cirrhosis and cancer (Hotamisligil, 2006).

An increased prevalence of steatosis and insulin resistance in HCV patients is well established and has prognostic implications, as it is associated with faster progression of fibrosis and a poorer response to treatment. In HCV patients

infected with genotypes 1 and 2, steatosis presents in general with concomitant obesity or other features of the metabolic syndrome, but this association is weak in genotype 3 patients. Genotype 3 is thought to induce steatosis in a direct fashion, as steatosis in these patients correlates with viral load, and reverses with response to treatment (Negro, 2006). HCV is thought to induce steatosis by impairing secretion and degradation, and increasing the neosynthesis of lipids. The HCV core protein, which localizes to the surface of lipid droplets and mediates the viral assembly in close conjunction with cellular fatty acid metabolism (Miyanari *et al.*, 2007), and also some HCV non-structural proteins, have all been shown to interfere with vLDL secretion (Wetterau *et al.*, 1997; Domitrovich *et al.*, 2005). HCV infection has also been associated with an upregulation of the neosynthesis of lipids (Waris *et al.*, 2007), inhibition of fatty acid oxidation (Dharancy *et al.*, 2005), and increased release of fatty acids from fat cells (Negro, 2006). Overall, the effects of HCV proteins on lipid synthesis, secretion and oxidation seem to be most potent in the context of genotype 3, but also occur in the context of other genotypes.

The development of severe steatosis and HCC was shown in PPARalpha-homozygous mice with liver-specific transgenic expression of the core protein gene, while tumours were not observed in the other transgenic mouse genotypes. This result suggested that persistent activation of PPARalpha, a central regulator of triglyceride homeostasis, is essential for the pathogenesis of hepatic steatosis, and HCC induced by HCV infection (Tanaka *et al.*, 2008).

Besides changes in the lipid metabolism, core and several of the non-structural HCV proteins induce systemic oxidative stress and related signalling by various mechanisms (Tardif *et al.*, 2005).

With respect to insulin resistance, all HCV genotypes have been shown to interfere with glucose homeostasis, often at early stages of infection (Negro, 2006), but the underlying mechanisms and the degree of insulin resistance seem to be again genotype-dependent. HCV has been shown to interfere with insulin signalling by proteasomal degradation of the insulin receptor substrates, (IRS)-1 and –2 (Aytug *et al.*, 2003).

The feedback circle between steatosis, insulin resistance and oxidative stress is an important denominator for disease progression in NAFLD as well as viral hepatitis, and induces tissue damage and inflammation and consequently, activation of hepatic stellate cells (HSCs). Activated HSCs become responsive to both proliferative and fibrogenic cytokines, and undergo epithelial-to-mesenchymal transdifferentiation (EMT) into contractile myofibroblast-like cells, that synthesize extracellular matrix (ECM) components, which accumulate over time to form fibrous scars, or 'fibrosis'. Ultimately, nodules of regenerating hepatocytes become enclosed by scar tissue, which defines cirrhosis. Activation of HSCs is regulated by products and effectors of oxidative stress and growth factors, cytokines, adipokines, and chemokines. The cytokine TGF-β, a potent inhibitor of epithelial cell growth and tumour suppressor, can also exert pro-oncogenic functions, and is a key regulator of EMT. Importantly, recent findings imply that TGF-β induces EMT not only in HSCs but possibly also in hepatocytes (Matsuzaki *et al.*, 2007). TGF-β signalling is upregulated in fibrotic HCV patients, and stimulates ECM deposition and accumulation. Insulin resistance may link fibrosis and steatosis, as it stimulates HSCs to deposit ECM. Several signalling cascades are implicated and modulated during fibrogenesis, including SMADs, PI3K-Akt and various MAPK pathways, such as p38 and JNK. While SMADS are indispensable for the EMT process, TGF-β signalling via SMAD synergizes with other signalling pathways to mediate pro-oncogenic EMTs. JNK activation by the pro-inflammatory cytokine interleukin-1β can shift TGF-β signalling away from a tumour-suppressive to a pro-oncogenic

profile with augmented fibrogenesis, increased cell motility, and transactivation of cell cycle regulatory genes (Matsuzaki *et al.*, 2007). Thus, in the context of chronic inflammation, the interplay between endoplasmic reticulum/oxidative stress, steatosis and insulin resistance induces a pro-oncogenic microenvironment that drives fibrogenic processes and genomic instability; and even though HCV has been reported to display direct transforming capacities, the liver microenvironment is thought to determine significantly the transformation process because HCC develops in chronic HCV infection only over long periods of time.

So far, it has not been possible to correlate hepatocarcinogenesis with a consistent pattern of proto-oncogene activation, but several growth factor signalling axes are frequently found to be dysregulated, including insulin-growth factor (IGF), hepatocyte growth factor, Wnt, TGF-α/EGF and TGF-β signalling (Breuhahn *et al.*, 2006; Levrero, 2006). The interplay between these various pathways and their respective roles and contributions to the development of HCC remain to be unravelled.

4.3.3 Role of HCV in lymphomas and other tumours

The mechanisms by which lymphoma is induced by HCV remains the subject of debate. Several clinical studies have shown that the HCV genome may be detected in peripheral lymphoid cells as well as dendritic cells (Bain *et al.*, 2001). However, evidence of true viral genome replication in extrahepatic sites is still lacking.

Some early studies showed that HCV may infect cultured peripheral blood mononuclear cells *in vitro* (Shimizu *et al.*, 1998), but these observations were not confirmed by other groups.

Few studies showed that the HCV genome sequence from extrahepatic isolates may cluster differently from liver isolates providing indirect evidence for viral replication in these cells (Roque-Afonso *et al.*, 2005).

Clinical studies have shown that HCV eradication by pegylated interferon and ribavirin treatment may lead to the cure of cryoglobulinemia, a B-cell proliferation disorder, and to the regression of HCV-associated splenic lymphoma (Hermine *et al.*, 2002).

Several non-exclusive hypotheses have been discussed regarding the transforming role of HCV in the context of lymphoma: 1) antigen-driven proliferation induced by continuous activation of B cells (Suarez *et al.*, 2006); 2) a direct role of HCV replication and expression in infected B cells.

Further molecular and cellular biology studies are warranted to decipher the mechanisms of HCV-induced lymphomas.

Regarding the role of HCV in the development of cholangiocarcinoma, both clinical evidence and strong experimental data are lacking.

4.4 Interaction between HCV and environmental agents

Regarding interactions between HBV and HCV please refer to the *Monograph* on HBV in this volume.

4.5 Animal models for HCV-associated cancers

Chimpanzees and tree shrews do not, or only partially, develop HCV-associated pathologies upon infection. And given the long delay with which HCC develops in chronic hepatitis, these models are unsuitable to study HCV-induced HCC in the first place. In the absence of animal models that develop HCC in the context of an HCV infection, various groups have described the use of mouse models. Mice expressing HCV replicons, polyproteins or the single HCV proteins alone or in combination, using various

liver specific promoters, have been described by many groups (Levrero, 2006; McGivern & Lemon, 2009).

To date, studies using transgenic animals expressing HCV cDNA suggest that HCV proteins are not directly cytopathic (Kawamura *et al.*, 1997; Pasquinelli *et al.*, 1997; Wakita *et al.*, 1998). Only three different HCV core transgenic lines have been shown to develop liver steatosis and HCC (Moriya *et al.*, 1998, 2001), and one group has been able to demonstrate that upon HCV polyprotein expression, the rate of liver cancer in transgenic mice increases in the absence of intrahepatic inflammation, suggesting a metabolic or genetic host susceptibility for HCV-associated HCC (Lerat *et al.*, 2002).

NS5A transgenic mice, despite the abundant interactions of NS5A with host-cell factors, do not have any significant phenotype (Majumder *et al.*, 2002, 2003).

4.6 HCV, host immune system, and genetic susceptibility

While many studies have been reported regarding the role of the humoral and cellular responses in the control of HCV infection, as well as micro-array analysis of primary liver tumours showing differential expression of many cellular genes in the tumours, no relevant data are available at the time of writing concerning specific immune or genetic mechanism involved in HCV-induced HCC.

4.7 Synthesis

Although there is strong evidence that HCV is one of the leading causes of HCC, there is still much to understand regarding the mechanism of HCV-induced transformation. While liver fibrosis resulting from long-lasting chronic inflammation and liver regeneration resulting from immune-mediated cell death are likely factors contributing to the development of HCC, the direct role of HCV proteins remains to be determined. Many *in vitro* studies have shown that HCV expression may interfere with cellular functions that are important for cell differentiation and cell growth. However, most studies were performed in artificial study models which can only give clues for potential mechanisms that need to be confirmed in more relevant models. Furthermore, the difficulty to localize HCV proteins as well as infected cells *in vivo* in the liver of infected patients contribute to the complexity of our current understanding.

For all these reasons, at the time of writing, the current view is that there is moderate experimental evidence for a direct oncogenic role of HCV. Further studies are warranted to clarify these issues.

5. Evaluation

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

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

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