FINAL

Report on Carcinogens Background Document for

Hepatitis C Virus

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FOREWORD

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of all substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (DHHS) has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP) who prepares the Report with assistance from other Federal health and regulatory agencies and non-government institutions.

Nominations for listing in or delisting from the RoC are reviewed by a formal process that includes a multi-phased, scientific peer review and multiple opportunities for public comment. The review groups evaluate each nomination according to specific RoC listing criteria. This Background Document was prepared to assist in the review of the nomination of Hepatitis C Virus. The scientific information in this document comes from publicly available, peer reviewed sources. Any interpretive conclusions, comments or statistical calculations, etc made by the authors of this document that are not contained in the original citation are identified in brackets [ ]. If any member(s) of the scientific peer review groups feel this Background Document does not adequately capture and present the relevant information they will be asked to write a commentary for this Background Document that will be included as an addendum to the document. In addition, a meeting summary that contains a brief discussion of the respective review group's review and recommendation for the nomination will be added to the Background Document, also as an addendum.

A detailed description of the RoC nomination review process and a list of all nominations under consideration for listing in or delisting from the RoC can be obtained by accessing the NTP Home Page at http://ntp-server.niehs.nih.gov. The most recent RoC, the 10th Edition, was published in 2002 and may be obtained by contacting the NIEHS Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov (800-315-3010).

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

United States Department of Health and Human Services
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Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Executive Summary

Introduction

Hepatitis C virus (HCV), a flavivirus, causes most non-hepatitis B parenterally transmitted viral hepatitis. HCV is an enveloped virus with a positive-sense RNA genome consisting of 9,600 nucleotides. The genome encodes a polyprotein of about 3,000 amino acids, which is cleaved into the mature virion structural and nonstructural proteins. The structural proteins consist of the core (nucleocapsid) protein and two specific glycoproteins that make up the lipid envelope (E1 and E2). The nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B), perform a number of enzymatic functions, including protease, nucleotide triphosphatase, RNA helicase, and RNA polymerase activity, that are essential to protein processing and RNA replication.

Replication of HCV often results in random nucleotide errors that are not corrected by the RNA polymerase because it lacks a proofreading function. As a result, HCV strains show extensive genomic heterogeneity; however, the variability is not uniformly distributed over the genome. HCVs can be divided into six phylogenetically distinct groups of genotypes designated as clades based on their variation in the well-conserved regions of the genome. Within the clades, multiple subtypes (genotypes) have been defined. All HCV types identified have the potential to cause serious liver disease.

The chimpanzee is the only established animal model that both is permissive for HCV infection and develops a disease syndrome mimicking that seen in humans. Transgenic mouse models producing HCV proteins or human major histocompatibility complex molecules and immunodeficient mice made chimeric with human liver cells continue to be pursued as tools to study HCV pathogenesis, to test vaccine strategies, and to develop antiviral agents.

HCV was nominated for possible listing in the Report on Carcinogens by the National Institute of Environmental Health Sciences on the basis of the International Agency for Research on Cancer (IARC) classification of HCV as carcinogenic to humans (Group 1) based on a finding of sufficient evidence of carcinogenicity in humans (hepatocellular carcinoma).

Human Exposure

Detection methods. HCV infection is usually assessed by detection of antibodies against HCV proteins or by detection of HCV RNA. Anti-HCV antibodies are detected by serological assays, which have undergone a series of improvements resulting in greater sensitivity and specificity. HCV RNA is usually detected by polymerase chain reaction-based tests.

Prevalence and incidence. The worldwide prevalence of HCV seropositivity is approximately 3% (170 million individuals); however, the prevalence varies geographically. Reported prevalence rates are low (less than 0.5%) in Western Europe, North America, Australia, most of Central and South America, and parts of Africa; intermediate (1% to 5%) in Eastern Europe, the Middle East, the Mediterranean, and

parts of Africa and Asia; and highest (17% to 26%) in Egypt. In the United States, approximately 3 to 4 million individuals are infected with HCV. The incidence of HCV infection in the United States has decreased in the last decade from 180,000 to 30,000.

Risk factors and transmission. The major route of HCV transmission is through contaminated blood. The major risk factor for infection is illegal intravenous drug use, which accounts for 60% of acute HCV infections in adults. Since the initiation of screening of blood and blood products in the 1990s, blood transfusion has accounted for only a small percentage of adult HCV cases (~3%). Other routes of transmission include sexual, perinatal, familial (at low rates), and through healthcare practices, including transmission by contaminated equipment or supplies, from patient to patient (at low rates), and through occupational exposure (at low rates).

Natural history and treatment. HCV is the leading cause of known liver disease in the United States. HCV infection can result in chronic hepatitis, cirrhosis, or liver cancer. Currently, HCV is treated by interferon-related therapies, and no vaccine is available.

Human Cancer Studies

A 1993 IARC Working Group evaluated the carcinogenic risk to humans of chronic HCV infection and concluded that “the agent is carcinogenic to humans” based on the finding of significantly increased relative risk of hepatocellular carcinoma (5 to >100) among anti-HCV-positive subjects. Numerous cohort and case-control studies published since the IARC review and conducted in populations differing by race-ethnicity and geography have further strengthened the recognized association between chronic HCV infection and development of hepatocellular carcinoma. These studies included a meta-analysis of 32 studies published before and after the IARC review, which calculated an odds ratio (OR) of 11.5 (95% confidence interval = 9.9 to 13.3). The recent studies generally used relatively sensitive and specific serological markers to assess chronic HCV infection, and many included information on potential confounders, such as use of alcohol and tobacco. These studies unequivocally show that the strong association between HCV and hepatocellular carcinoma is independent of hepatitis B virus (HBV) infection (i.e., the association is clearly present when subjects are confined to non-carriers of HBV) and essentially unaltered after adjustment for nonviral risk factors for hepatocellular carcinoma.

A number of recent studies have examined the possibility that the various HCV genotypes may differ in their carcinogenic potencies. Although the results are not entirely consistent, the overall evidence supports the hypothesis that HCV genotype 1b is more strongly associated with hepatocellular carcinoma than are other HCV genotypes.

Consistent and abundant data support a synergistic effect of HBV and HCV coinfection on risk of hepatocellular carcinoma. The meta-analysis reported that individuals with biomarkers for both viruses were at an approximately six-fold greater risk (OR ~135) than individuals who had biomarkers for only one hepatitis virus (OR ~24 for HCV alone, and OR ~20 for HBV alone). There also is evidence that heavy alcohol intake acts

as a cofactor to enhance the risk of hepatocellular carcinoma in HCV-infected individuals.

A number of recent case-control studies and one cohort study have linked HCV infection to increased risk of B-cell lymphoma. Many of these studies had relatively small sample sizes, and all were hospital based. Better-designed, population-based case-control and cohort studies are needed to confirm these preliminary findings.

Studies in Experimental Animals

Experimental animal studies of HCV are limited because of the narrow host range. The chimpanzee and tree shrew are the only animals that are susceptible to infection with HCV. However, in recent years, the cloned HCV genome has been used to develop transgenic animal models in which to study HCV pathogenesis. Hepatocellular carcinoma has been reported in one chimpanzee infected with HCV for seven years, but not in HCV-infected tree shrews. Hepatocellular carcinoma also developed in a few lines of transgenic mice (primarily males) producing either the HCV core protein or low levels of the complete HCV polyprotein.

Other Relevant Data

Pathogenesis. Most individuals infected with HCV develop chronic hepatitis, which is associated with chronic liver injury and inflammation. Liver injury appears to be a result of the host immune reaction to the virus rather than damage from the virus itself. Chronic infection usually results in progressive hepatic fibrosis, which may progress to cirrhosis and other disease states.

Potential mechanisms of carcinogenesis. The mechanisms of HCV-related hepatocarcinogenesis have not been elucidated. HCV may cause cancer directly or indirectly as a result of hepatic inflammation and regeneration associated with chronic hepatitis. Because HCV is an RNA virus, and thus does not integrate into the host DNA, direct mechanisms would most likely involve production of viral protein. The HCV core protein is the current leading suspect, based on its role in regulating cellular promoters and proto-oncogenes and on studies in transgenic mice. *In vitro* studies have reported that the core protein cooperates with *ras* to transform primary rat embryo fibroblasts to a tumorigenic phenotype. The HCV core protein also induces tumors in some but not all lines of transgenic mice. The roles of other viral proteins in hepatocarcinogenesis remain largely unexplored. HCV-related liver cancer almost always arises in the presence of cirrhosis, suggesting the importance of indirect mechanisms such as inflammation, fibrosis, and hepatocyte regeneration in cancer development. The prevailing hypothesis of how cirrhosis results in hepatocellular carcinoma is that dysplastic nodules develop within the cirrhotic liver. The pathway of hepatocarcinogenesis related to HCV infection appears to be different from that associated with HBV infection. HBV-associated liver cancer is characterized by genetic instability, whereas several but not all studies have reported an association between β-catenin gene mutations and HCV-associated liver cancer; however these studies were based on small numbers of tumors. The interplay

between these mutations and underlying liver disease (i.e., presence, duration, and severity of underlying liver disease) remains to be examined.

Epidemiologic studies have shown that there is a synergistic risk of hepatocellular carcinoma in individuals chronically infected with both HCV and HBV, but the mechanisms of this interaction is unknown. Coinfection with HBV may exacerbate the liver disease activity from HCV, and thus increase hepatocellular carcinoma risk. Nonviral cofactors that also may increase the risk of liver cancer in HCV patients include alcohol, smoking, and betel-quid chewing.

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1 Introduction

With the development of assays specific for the hepatitis A and B viruses and their antibodies, it became clear that many cases of transmissible hepatitis were not related to either of these classic viruses. These cases were termed non-A, non-B hepatitis. It was shown, in fact, that most transfusion-associated cases of hepatitis were not due to the hepatitis B virus (HBV), but were caused by some other agent (Feinstone *et al.* 1975, Prince *et al.* 1974). After a search of about 15 years, a new virus termed hepatitis C virus (HCV) was identified as the agent responsible for most transfusion-associated non-A, non-B hepatitis (Choo *et al.* 1989). A different agent, now called hepatitis E virus, had been shown to be responsible for sporadic and outbreak-associated cases of non-A, non-B hepatitis, with a transmission and epidemiology similar to that of hepatitis A (Balayan *et al.* 1983). HCV was originally identified by use of molecular cloning technology in which a cDNA expression library prepared from RNA extracted from a persistently infected chimpanzee was screened with serum from non-A, non-B hepatitis patients as a source of specific antibody. The virus was shown to be a positive-sense, single-stranded RNA virus of the Flaviviridae family.

HCV causes most parenterally transmitted non-B viral hepatitis. It is estimated that at least 170 million people worldwide are infected with HCV and between three and four million in the United States alone (Alter 1999, Alter *et al.* 1999, Mast *et al.* 1999).

HCV infections often result in relatively mild acute disease. However, what makes hepatitis C such an important problem is its propensity to cause persistent infections, which are estimated to occur in at least 75% of infected persons. Chronic infections with HCV tend to progress slowly but may eventually result in significant chronic liver disease, including cirrhosis and hepatocellular carcinoma.

HCV was nominated for possible listing in the Report on Carcinogens by the National Institute of Environmental Health Sciences (NIEHS) on the basis of the International Agency for Research on Cancer (IARC) classification of HCV as carcinogenic to humans (Group 1) based on a finding of sufficient evidence of carcinogenicity in humans (hepatocellular carcinoma).

1.1 Taxonomy

HCV is a member of the Flaviviridae family of viruses, which comprises three distinct genera (see Figure 1-1): (1) the classic flaviviruses, such as yellow fever, dengue, and Japanese encephalitis, (2) the pestiviruses of animals, including bovine viral diarrhea virus and classic swine fever virus, and (3) the hepaciviruses, of which hepatitis C is the sole example. The GB viruses (GBV-A, GBV-B, and GBV-C) clearly are flaviviruses, but it has not yet been determined whether they should be in a genus of their own. GBV-A and GBV-C (the latter also called hepatitis G virus, or HGV) are closely related to each other but are not strictly hepatotropic. These viruses have a composition similar to that of HCV but lack a defined coding region for a core protein (Beames *et al.* 2001, Robertson *et al.* 1998). GBV-B causes hepatitis in several species of New World monkeys, and its sequence is more closely related to that of HCV than to that of either GBV-A or GBV-C

(see Figure 1-1). GBV-B is being considered for inclusion in the genus *Hepacivirus* (Leary *et al.* 1996, Yanagi *et al.* 1997).

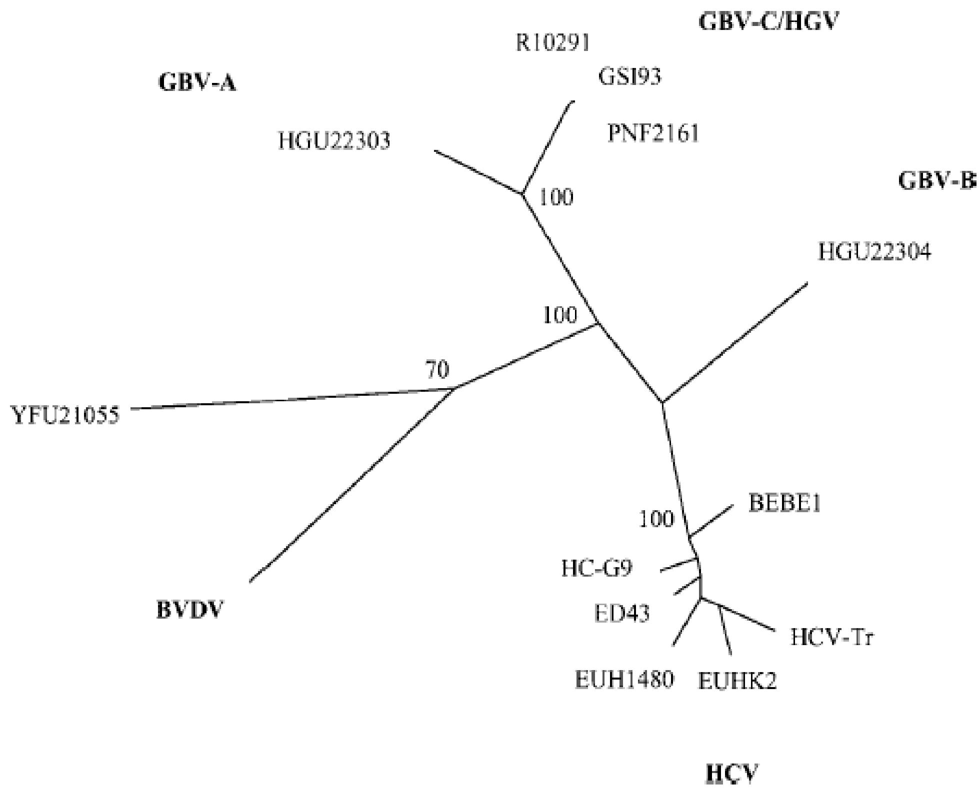

Figure 1-1. Phylogenetic tree of the Flaviviridae

Source: Robertson *et al.* 1998. Reprinted with permission.

Note: This figure illustrates the relationship of the hepaciviruses (HCV) to the pestiviruses (BVDV) and the flaviviruses (yellow fever virus [YFU21055]). The GB viruses A, B, and C also are shown for their relationship to HCV.

1.1.1 Classification

Hepatitis C virus has a genomic organization and polyprotein hydrophobicity profile similar to those of the pestiviruses and classic flaviviruses (Choo *et al.* 1991) and has been classified as a separate genus, *Hepacivirus*, in the family Flaviviridae (Robertson *et al.* 1998). The *Hepacivirus* genus includes only HCV, but several related viruses of humans and other primates may eventually be included (Robertson *et al.* 1998) (see Figure 1-2).

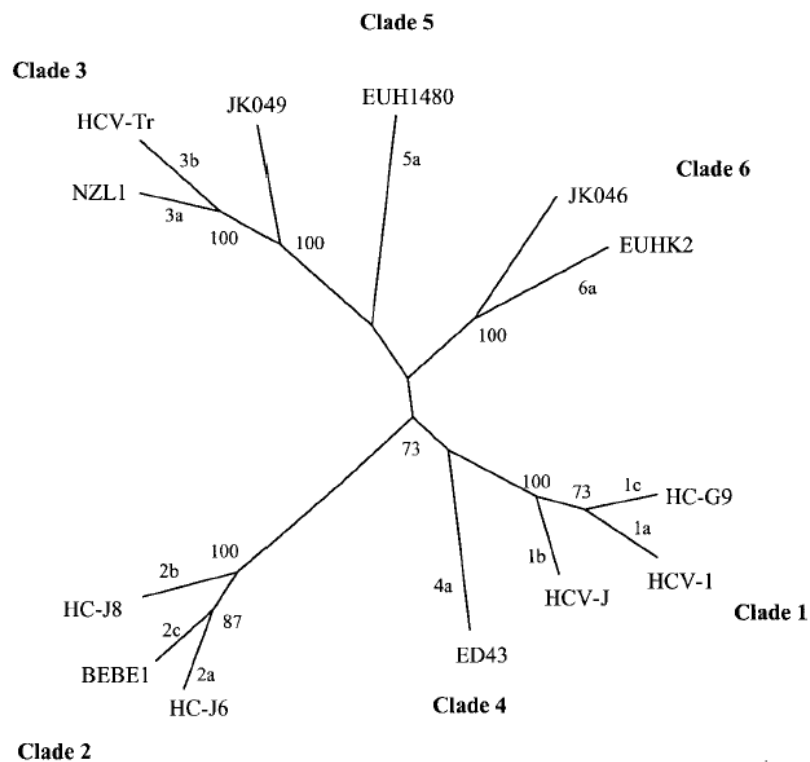

Figure 1-2. A phylogenetic tree showing the the various HCV genotypes classified into clades (groups of genotypes sharing a common ancestor).

Source: Robertson *et al.* 1998. Reprinted with permission.

Like the other flaviviruses, the HCV viral particle is approximately 50 nm in diameter (He *et al.* 1987, Shimizu *et al.* 1996a) and consists of an envelope derived from host membranes, into which are inserted the virally encoded glycoproteins (E1 and E2), surrounding a nucleocapsid and a positive-sense single-stranded RNA genome of

approximately 9,600 nucleotides (Choo *et al.* 1991). The HCV genome contains highly conserved untranslated regions (UTRs) at both the 5' and 3' termini (Han *et al.* 1991, Kolykhalov *et al.* 1996, Tanaka *et al.* 1996), which flank a single open reading frame (ORF) encoding a polyprotein of approximately 3,000 amino acids (Choo *et al.* 1991, Rosenberg 2001, Takamizawa *et al.* 1991). This protein is processed co- and post-translationally by cellular and viral proteases to produce the specific viral gene products. The structural proteins (the core protein and two putative envelope proteins, E1 and E2) are located in the N-terminal quarter, and the nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) in the remaining portion of the polyprotein (Grakoui *et al.* 1993a, Hijikata *et al.* 1991, Rosenberg 2001).

1.1.2 Genotypes

HCV strains show extensive genome heterogeneity, although this variability is not uniformly distributed over the genome. Relatively well-conserved regions of the HCV genome (core, E1, and NS5) have been studied extensively and used as the basis for genotype classification. All currently known isolates of HCV can be divided into six phylogenetically distinct groups designated as clades 1 to 6, consisting of the previously identified genotypes (see Figure 1-2) (Robertson *et al.* 1998). Within the clades, multiple subtypes have been defined (e.g., genotypes 1a, 1b, 1c) (Bukh *et al.* 1995, Simmonds 1995, Simmonds *et al.* 1993a).

Antibodies to specific regions within the core or NS4 proteins have been used to distinguish among some HCV genotypes, as the amino acid sequence varies at the sites of reactive B-cell epitopes (Simmonds *et al.* 1993b). However, these serological differences do not equate with distinct serotypes based upon antibody neutralization as generally defined for most viruses. To date, no classification of HCV exists that is based on serotyping. These antibody data for HCV only correlate with genotype and provide a relatively simple and quick method to determine the genotype of a virus. Other typing methods include (1) restriction fragment length polymorphism (RFLP) of the 5' UTR (Simmonds *et al.* 1993c), (2) reverse dot-blot hybridization analysis of 5' UTR, or line probe assay (Stuyver *et al.* 1996), and (3) nested polymerase chain reaction (PCR) amplification of the core gene using type-specific primers (Okamoto *et al.* 1992). PCR amplification and sequence analysis remains the most reliable method for determining genotype differences among HCV isolates.

Through sequence analysis, as many as 11 genotypes had been defined, although not all of these were widely accepted as distinct from previously defined genotypes. A broader system was recently proposed in order to unify the nomenclature for HCV, define the requirements for classification, and accommodate isolates that did not fit into the scheme defined by Simmonds, which was based on phylogenetic analysis of only the NS5 region (Robertson *et al.* 1998, Simmonds *et al.* 1993a). Under the new system, the concept of six major groups of HCV based upon sequence homology remains, but these are distinguished phylogenetically and described as clades. Genotypes 1, 2, 4, and 5 are identified as clades 1, 2, 4, and 5, respectively; genotypes 3 and 10 become members of clade 3, and genotypes 6 through 9 and 11 are included in clade 6. Although this classification into clades has been accepted formally, the earlier genotype designations remain in common use and are used in this discussion.

Despite the discussions surrounding the classification of HCV sequences, clinical and virologic data have revealed few significant phenotypic differences among genotypes. It appears that patients infected with types 1a and 1b respond, on average, less well to interferon treatment and tend to experience more rapid progression to chronic liver disease than those infected with other genotypes (Bukh *et al.* 1995). It must be remembered that all types of HCV isolated to date have the potential to cause serious chronic liver disease.

1.2 Structure and virology

1.2.1 Virion structure

Viruses are basically protein packages containing the nucleic acid, which is the genetic material (genome) of the virus. Viruses can be generally classified by their type of genetic material (nucleic acid), either RNA or DNA, which can be single stranded or double stranded. Most (but not all) DNA viruses have double-stranded genomes, and most (but not all) RNA viruses have single-stranded genomes. Viruses also can be classified by their physical structure. The genome of most viruses is associated with a protein termed a nucleoprotein, which may bind around the viral genome or form a capsid structure, which may look like a ball with the nucleic acid inside. Simple viruses consist only of the capsid and nucleic acid, but others also acquire a lipid-containing envelope surrounding the nucleocapsid. During the replication process, this envelope is taken from one of the membranes of the cell in which the virus replicates, and the virus inserts virus-coded proteins into the envelope. These envelope proteins perform specific functions for the virus, such as attachment to new cells to initiate the replication cycle. HCV is an enveloped single-stranded RNA virus. Its RNA genome consists of approximately 9,600 nucleotides (the building blocks of the RNA chain). The genome is associated with the HCV core protein, which is the nucleocapsid protein, and the virus has an envelope into which two virus-specific proteins, E1 and E2, are inserted. All the viruses in the flavivirus family share a similar structure, in that they all are enveloped single-stranded RNA viruses with genomes of approximately 10,000 nucleotides.

Before HCV was identified, it was shown that the etiologic agent of non-A, non-B hepatitis could be inactivated by chloroform (Feinstone *et al.* 1983), indicating that the particles had a lipid-containing envelope, and filtration studies showed that the pathogen was between 30 and 60 nm in diameter (He *et al.* 1987). Low levels of virus in plasma samples and the lack of robust *in vitro* cultivation systems have made visualization of this virus difficult. However, enveloped virus-like particles have been identified by electron microscopy (Shimizu *et al.* 1996a) (see Figure 1-3). Spherical particles approximately 50 nm in diameter that reacted specifically with HCV antibodies were observed in thin-section electron micrographs of cells from an *in vitro* infected cell line. Most electron micrographs of putative HCV particles show not a well-defined core structure, but more of an amorphous electron-dense material within the viral envelope. However, electron micrographs (see Figure 1-4) in which optical rotation techniques have been used on purified, isolated cores have revealed particles with an icosahedral structure (Ishida *et al.* 2001).

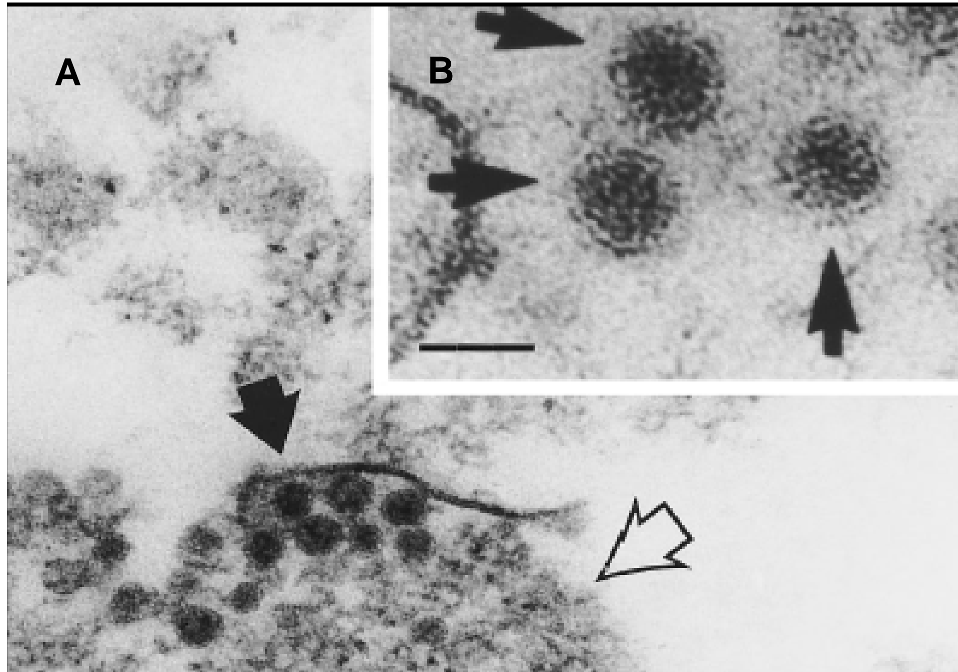

Figure 1-3. Thin-section electron micrograph of *in vitro* infected HPBALL cells harvested 25 days post inoculation with HCV

Source: Shimizu *et al.* 1996a. Reprinted with permission.

Note: (A) Virus-like particles associated with a cytoplasmic membrane or possibly a vesicle. (B) Higher magnification of cytoplasmic virus-like particles with outer envelope and an electron-dense core. Other studies showed immunologic identity of these particles with HCV. Bar = 100 nm.

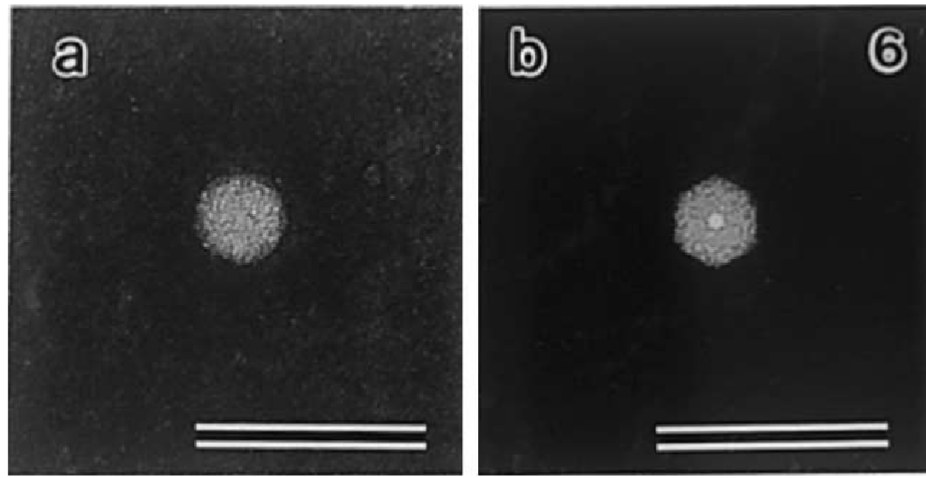

Figure 1-4. Electron micrographs of isolated HCV cores purified from HCV positive plasma

Source: Ishida *et al.* 2001. Reprinted with permission.

Note: Icosahedron-like particles 35 to 40 nm in diameter were observed by an optical rotation (n = 6) technique, Bars = 100 nm.

Sucrose density gradient analyses of HCV-positive sera and plasma have isolated both low-density (1.06 to 1.13 g/mL) and high-density (1.17 to 1.25 g/mL) fractions containing HCV RNA (Hijikata *et al.* 1993a, Kanto *et al.* 1994, Thomssen *et al.* 1993). It has yet to be established whether these fractions represent intact virions or forms of subviral material containing HCV RNA. HCV in these low-density fractions has been associated with lipoproteins of low density (LDL), intermediate density, and very low density (VLDL) (Andre *et al.* 2002). This association with plasma lipoproteins is likely to be responsible for the observed density of 1.06 g/mL, which is unusually low even for an enveloped RNA virus such as HCV and is close to that of plasma LDLs (Andre *et al.* 2002, Hijikata *et al.* 1993a). The higher-density fractions (1.1 to 1.18 g/mL) possibly represent free virus or particles complexed with immunoglobulin (Choo *et al.* 1995, Hijikata *et al.* 1993a). Potentially, association with LDL can confer two advantages to the virus: (1) particles may be protected from antibody-mediated neutralization, and (2) virions may gain entry into cells via the cellular LDL receptor. These data argue in favor of a protective association between HCV virions and LDL, and recent reports suggest that the LDL receptor can mediate uptake of HCV; however, it is unclear whether this uptake leads to productive infection (Agnello *et al.* 1999, Monazahian *et al.* 1999).

1.2.2 Virologic and molecular characteristics

The genome of HCV is positive-sense, single-stranded RNA approximately 9.6 kb in length. It contains a single long ORF coding for a polyprotein of between 3008 and 3037 residues (see Figure 1-5). The ORF is flanked by a 5' and a 3' UTR. Recently, it has been shown that frame shifting within the core coding region can result in translation of a protein in a different reading frame (Xu *et al.* 2001). Replication is believed to occur entirely within the cytoplasm and is closely associated with cytoplasmic membranes. Most of the reports regarding genome function and the processing of HCV proteins are based upon *in vitro* studies in mammalian, insect cell, or bacterial expression systems. Kolykhalov *et al*. (2000) demonstrated an essential requirement for the enzymatic activities of the NS2/3 protease, the NS3-4A serine protease, the NS3 nucleotide triphosphatase (NTPase)/helicase, and the NS5B polymerase in chimpanzees *in vivo*. Inoculation with full-length, known infectious HCV RNA that carried specific mutations engineered to remove these enzymatic activities did not result in productive infections. In addition, it has been shown that elements within the 3' UTR of the HCV genome are required for successful replication, based on infectivity in chimpanzees (Kolykhalov *et al.* 2000, Yanagi *et al.* 1999). These observations suggest that the data obtained from *in vitro* systems provide a good representation of events occurring during productive viral infection.

Figure 1-5. Diagram of HCV genomic RNA and its translation products

Note: The diagram indicates the structure of the polyprotein and its processing by cellular signal peptidases and virally encoded proteinases (NS2/3 and NS3/4A). The approximate sizes of the mature viral proteins (p) and glycoproteins (gp) are indicated. See Table 1-1 for nucleotide and amino acid locations of the mature viral proteins.

1.2.2.1 The 5' untranslated region

The complete 5' UTR consists of 341 nucleotides (Han *et al.* 1991); its proposed secondary structure (Honda *et al.* 1999a) is shown in Figure 1-6. This region is one of the most conserved portions of the HCV genome (Bukh *et al.* 1992, Smith *et al.* 1995), and although nucleotide variations characteristic of different HCV types exist and have been used in PCR-based genotyping assays (Smith *et al.* 1995), the overall secondary structure is highly conserved. Several groups have confirmed that the 5' UTR contains an internal ribosome entry site (IRES) that can direct translation in a cap-independent manner (Fukushi *et al.* 1994, Reynolds *et al.* 1996, Wang *et al.* 1993). It has now been shown by cryoelectron microscopy that this IRES binds physically to the 40S ribosomal subunit, altering its conformation and locking the RNA in the ribosomal decoding center (Spahn *et al.* 2001).

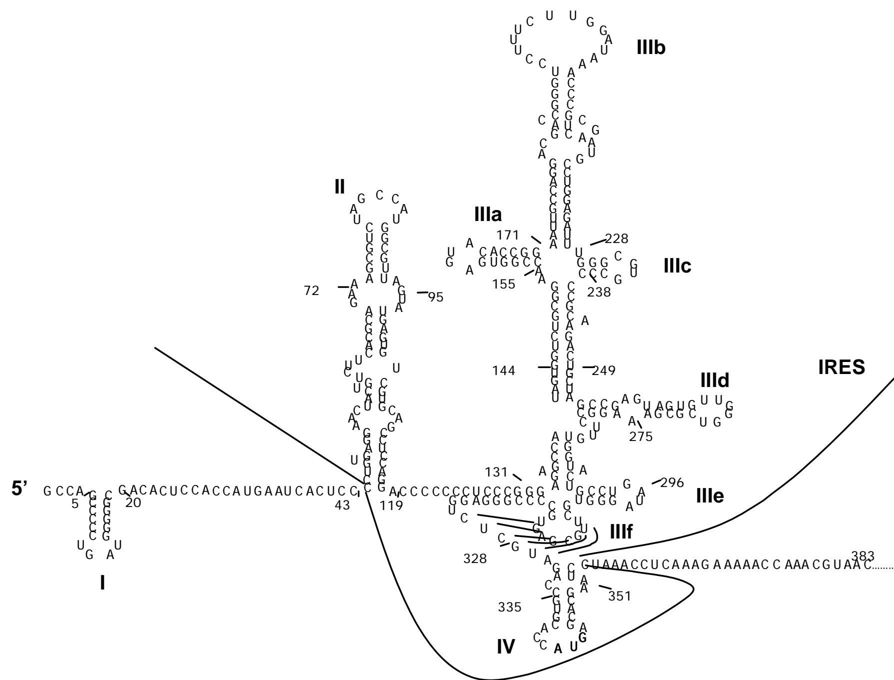

Figure 1-6. A model showing the secondary and tertiary structure of the HCV 5' untranslated region (H strain, or genotype 1a) indicating the individual stem-loop structures important for the IRES function

Source: Honda *et al.* 1999a. Reprinted with permission.

Note: The minimal region required for IRES function (nucleotides 43 to 354) is indicated. The structure was developed by both computer prediction and experimental data. The AUG start codon is in bold type.

The IRES occupies most of the HCV 5' UTR, as shown in Figure 1-6, conservation of the sequence downstream of the initiating AUG (the transcription initiation codon) is required for efficient IRES function. In the genome, a coding sequence of approximately 12 to 30 nucleotides from the core region is sufficient for this IRES function (Honda *et al.* 1996a, Reynolds *et al.* 1996), but some reporter gene sequences can be substituted (Fukushi *et al.* 1994, Honda *et al.* 1996b). It has been suggested that specific sequences in this region downstream of the initiating AUG contribute to IRES function through RNA-RNA interaction (Honda *et al.* 1999a, 1996b). The 5' UTRs from different genotypes have been shown to direct translation with different efficiencies (Collier *et al.* 1998, Honda *et al.* 1999b), which may depend on subtle sequence variations within this region that affect RNA-RNA interactions or RNA-protein interactions. Several cellular proteins have been shown to bind the 5' UTR of HCV and to play functional roles in HCV internal initiation (Ali and Siddiqui 1997, Hahm *et al.* 1998, Spangberg and Schwartz 1999), including polypyrimidine tract-binding protein and the eukaryotic translation initiation factor eIF3 (Ali and Siddiqui 1995, Buratti *et al.* 1998). However, the precise mechanism of translational control in HCV infection is still to be determined.

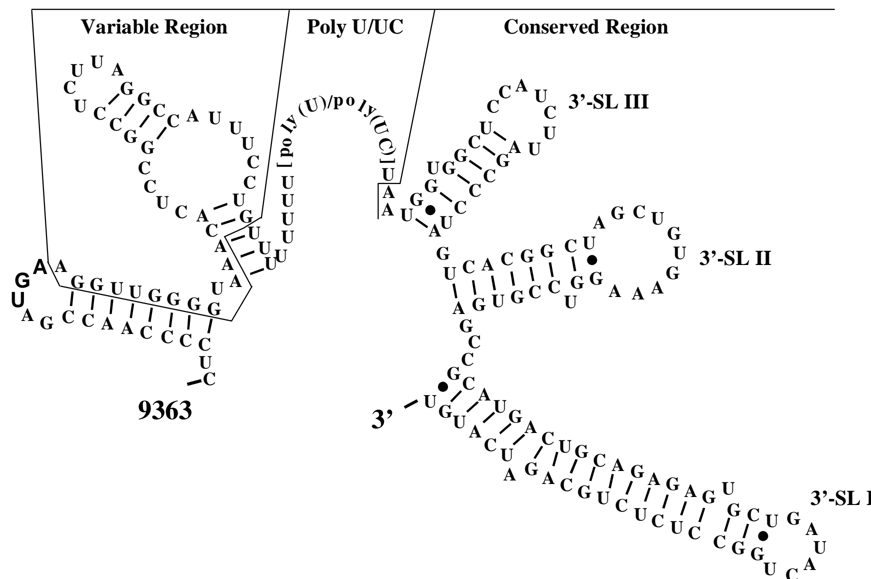

Figure 1-7. Computer-predicted secondary structure of the HCV 3' UTR (H strain, or genotype 1a) with limited upstream sequence

Source: Kolykhalov *et al.* 1996, as modified by S. Feinstone. Reprinted with permission

Note: The stem-loop (SL) structure and individual regions are labeled. The polyprotein open reading frame UGA termination codon located at nucleotide 9375 is in bold type.

The IRES element of HCV is a potential target for antiviral intervention. Anti-sense oligonucleotides, ribozymes, a small yeast inhibitory RNA, and inhibitors of eIF3 have been proposed for this purpose and have been shown to inhibit translation *in vitro* (Collier *et al.* 2002, Cornberg *et al.* 2001, Das *et al.* 1998, Macejak *et al.* 2001).

1.2.2.2 The 3' untranslated region

Following the ORF stop codon is the 3' UTR, which includes, in the 5' to 3' direction, a variable region (of 40 nucleotides in HCV-H), a polyU tract of variable length, a polypyrimidine $C(U)_n$ stretch, and a highly conserved 98-base sequence, thought to represent the 3' terminus of the genome (Kolykhalov *et al.* 1996). Tanaka *et al.* (1996) had previously described the highly conserved 98-base 3' terminus in unspecified strains; however, these authors described a 29-nucleotide region which they referred to as a conventional 3' UTR, with sequence homology to a portion of the variable region described by Kolykhalov et al. (1996). Computer predictions of the secondary structure and cleavage analyses have shown that the region can form stable stem-loops (Blight and Rice 1997, Ito and Lai 1997, Kolykhalov *et al.* 1996) (see Figure 1-7 for an approximation), although the two upstream stem loops have not been confirmed experimentally. Specific interactions between the 3' UTR, in particular, the conserved 98-base region at the 3' terminus, and cellular proteins, including polypyrimidine tract-binding protein (Ito and Lai 1997, Tsuchihara *et al.* 1997), suggest that this region is involved in viral replication and possibly translation (Ito and Lai 1999). Studies in chimpanzees with infectious clones of HCV deletion mutants have shown that the poly(U/UC) region and the conserved 98-base region are critical for infectivity, although the variable region, or its secondary structure, is not (Kolykhalov *et al.* 2000). This finding further confirms the involvement of the proximal 3' region of the HCV genome in replication.

1.2.2.3 The viral proteins

The functional HCV proteins are generated from the polyprotein following co- and post-translational cleavage by cellular and viral proteases. The nucleotide and amino acid locations of each functional protein are listed in Table 1-1. The structural proteins are characterized by hydrophobic domains at the C-termini that are important for membrane association and subsequent cleavage by the signal peptidases, localized in the lumen of the endoplasmic reticulum (ER) (Grakoui *et al.* 1993b). Cleavage at the junction between NS2 and NS3 occurs autoproteolytically via a protease encoded by NS2 and the N-terminal portion of NS3 (Grakoui *et al.* 1993a, Hijikata *et al.* 1993b), and cleavages of the other downstream NS proteins are mediated by a distinct virus-encoded serine protease located in the N-terminal third of NS3, which uses NS4a as a cofactor (Bartenschlager *et al.* 1995a, Eckart *et al.* 1993, Grakoui *et al.* 1993c, Hijikata *et al.* 1993b).

Table 1-1. Features of the HCV genome and polyprotein products

Genome region/ protein	Nucleotide location[a]	Amino acid location of the mature product in the HCV polyprotein[a]	Approx. size by SDS PAGE[b]	Function(s)
5' UTR	1–341	–[c]	–[c]	Initiation of translation, replication[p]
C	342–857	1–191/179/182	p21/p19	Structural, encapsidation of viral RNA [p]
E1	915–1490	192–383	gp31*	Structural, receptor binding, cell entry[p]
E2	1491–2579	384–746	gp70*	Structural, receptor binding, cell entry[p]
E2-p7	1491–2768	384–809	gp70*	Unknown; possible precursor or structural function
p7	2580–2768	747–809	p7	Possible membrane calcium channel
NS2	2769–3419	810–1026	p21	Part of NS2-3 protease
NS3	3420–5312	1027–1657	p70	Part of NS2-3 protease, serine protease, helicase, NTPase
NS4A	5313–5476	1658–1711	p6	Co-factor for NS3 serine protease activity
NS4B	5477–6257	1712–1972	p27	Replicase component[p]
NS5A	6258–7600	1973–2420	p58	Replicase component[p]
NS5B	7601–9374	2421–3011	p68	RNA-dependent RNA polymerase
3' UTR	9375–~9621	–	–	Replication[p], packaging of viral genome[p]

Source: Major *et al.* 2001.

[a]Based on HCV-H strain(genotype 1a) nucleotide and amino acid sequence.

[b]Sodium dodecyl sulfate polyacrylamide gel electrophoresis.

[c]5' UTR contains several short ORFs; whether polypeptides are produced, or their possible functions, is unknown.

*Indicates that proteins are N-glycosylated.

[p]Designates putative function(s) based upon comparisons with other viruses.

1.2.2.4 The core region

The first 191 amino acids of the HCV polyprotein constitute the putative nucleocapsid, or core protein, with a molecular weight of 21 kDa to 23 kDa (Hijikata *et al.* 1991). This region is highly conserved (Bukh *et al.* 1994) and includes several T- and B-cell epitopes (Battegay *et al.* 1995, Goeser *et al.* 1994, Kita *et al.* 1995). Forms with different molecular weights, representing C-terminally truncated products, have been identified by SDS-PAGE analysis (Hijikata *et al.* 1991, Santolini *et al.* 1994, Yasui *et al.* 1998). The truncated form of 19 kDa to 21 kDa is thought to represent the mature protein (Liu *et al.* 1997, Yasui *et al.* 1998), terminating at amino acids 173–174 (Liu *et al.* 1997, Santolini *et al.* 1994). Some of these truncated forms have been identified in the nucleus (Lo *et al.* 1995, Suzuki *et al.* 1995). The basic nature of the core N-terminus, which contains several putative nuclear localization signals and a DNA binding motif (Chang *et al.* 1994), may result in translocation to the nucleus; however, clinical data have not indicated the presence of the core in the nuclei of infected liver cells (Barba *et al.* 1997). The RNA binding activity has been localized to the N-terminus, amino acids 1–75 (Santolini *et al.* 1994); this sequence is characterized by a cluster of basic residues showing a high degree of conservation, suggesting an important biological function. The core protein has been shown to interact *in vitro* with several biologically active cellular proteins, such as tumor necrosis factor alpha (TNF-α) and nuclear factor kappa B (NF-κB) (Ray *et al.* 1998, 2002,), performing multiple functions in cells including transactivation and suppression (Marusawa *et al.* 1999, You *et al.* 1999). These numerous reports have implications for possible adverse effects of the core protein on cellular functions, which may contribute to pathogenesis and possibly to neoplastic transformation.

Recently, studies have reported that the core region may be translated in a second reading frame by ribosomal frame shifting beginning after codon 11 of the core coding region (Xu *et al.* 2001). Antibodies to this potential alternative reading protein have been detected in the sera of chronically infected patients. It also has been shown that the core coding region potentially may be translated in a third reading frame (Choi *et al.* 2002). The significance of these findings is not yet clear.

1.2.2.5 The envelope region

The glycoproteins E1 (gp31) and E2 (gp70) (the putative viral envelope proteins) are targeted to the ER by amino-terminal signal peptides and are N-terminally glycosylated at 5 or 6 and 11 sites, respectively (Dubuisson *et al.* 1994, Grakoui *et al.* 1993a, Lin *et al.* 1994). It has been shown that the proteins are retained in the ER rather than being retrieved from the Golgi complex during processing through the C-terminal hydrophobic transmembrane domains (Cocquerel *et al.* 1999). Based on the apparent sequence of events by which the viral polyprotein is cleaved, it appears that two possible precursors for E2 are produced (see Figure 1-5 and Table 1-1), E2-NS2 and E2-p7 (Dubuisson *et al.* 1994, Grakoui *et al.* 1993a, Lin *et al.* 1994, Mizushima *et al.* 1994). Signalase cleavage at the p7 site is inefficient, resulting in the presence of two forms of the second envelope protein, E2 and E2-p7 (Lin *et al.* 1994, Mizushima *et al.* 1994), differing only in their C-termini (see Figure 1-5). Whether p7 is present and the role of the E2-p7 protein in viral particle formation are both unknown. However, it has recently been reported that p7 is an

integral membrane protein with two transmembrane domains, and it can hexamerize to form a transmembrane pore that probably functions as a calcium channel (Carrere-Kremer *et al.* 2002).

Kinetic studies show that E1 and E2 interact slowly to form a stable complex (Deleersnyder *et al.* 1997, Dubuisson *et al.* 1994, Grakoui *et al.* 1993a). Properly folded complexes require N-linked glycosylation (Choukhi *et al.* 1998, Meunier *et al.* 1999) and are noncovalently bonded into heterodimers (Deleersnyder *et al.* 1997, Dubuisson *et al.* 1994, Ralston *et al.* 1993); a proportion of disulfide-bonded complexes has been identified which is thought to represent misfolded structures (Deleersnyder *et al.* 1997, Dubuisson *et al.* 1994). The interaction seems to be totally membrane associated, as the proteins remain localized to the ER and are not transported to the cell surface; however, deletion of the E2 C-terminal hydrophobic domain results in secretion of this protein from cells (Michalak *et al.* 1997).

An unusually high degree of amino acid variation has been observed in the N-terminus of the E2 protein (Weiner *et al.* 1991). This hypervariable region, referred to as the HVR1, is located between amino acids 384 and 410 of the polyprotein (amino acids 1 and 27 of E2). Any one clinical isolate of HCV contains multiple, related HVR1 sequences (Nakajima *et al.* 1996, Sekiya *et al.* 1994), resulting in a population of quasispecies. HVR1 sequences may be much less related between isolates, but the HVR1 varies independently of HCV genotype. It should be stressed that no specific HVR1 sequence pattern has been associated with any particular genotype; therefore, this region cannot be used to distinguish among genotypes.

Observations that specific antibodies to HVR1 epitopes change during the course of a chronic infection suggest that this region is subject to immune pressure, with the potential for escape mutants (Forns *et al.* 1999). However, a consensus profile of HVR1 sequences indicated that there might be more cross reactivity between variants than previously thought (Puntoriero *et al.* 1998). These data provide important considerations for prophylactic vaccine development.

1.2.2.6 The NS2 region

The HCV proteins were named by analogy to flavivirus proteins. No protein similar to flavivirus NS1 was identified, and thus the nonstructural region begins with NS2. The NS2 protein is cleaved from the polyprotein at its N-terminus by a cellular signal peptidase and at its C-terminus by an HCV protease encoded by most of the NS2 region and part of the NS3 domain. This NS2-3 protease is believed to mediate autoproteolytic cleavage at the 2/3 site (Grakoui *et al.* 1993b, Hijikata *et al.* 1993b), appears to be zinc-dependent, and can function, although inefficiently, in *trans* (Grakoui *et al.* 1993b, Reed *et al.* 1995). The region required for processing at the 2/3 site in mammalian cells lies between residues 849 and 1207 of the HCV polyprotein (Grakoui *et al.* 1993c, Santolini *et al.* 1995). However, this cleavage event is not required for NS3-serine protease-dependent cleavages downstream, and mutations inactivating the NS3-serine protease have no effect on 2/3 cleavage. Residues His-952 and Cys-993 of the NS2 region are essential for the NS2/3 cleavage and are thought to be involved in zinc binding, while mutations of Cys-1123, Cys-1125, and Cys-1171 in the NS3 region significantly decrease

autoproteolytic activity (Hijikata *et al.* 1993c). *In vitro* studies on NS2-3 protease have shown that the efficiency of processing depends at least partially upon the presence of microsomal membranes (Grakoui *et al.* 1993b, Santolini *et al.* 1995), suggesting the involvement of a cellular cofactor in this event. The exact mechanism of autoproteolysis at the 2/3 site is yet to be determined. Alternative zinc protease or cysteine protease mechanisms have been suggested (Wu *et al.* 1998); however, further structural and biochemical studies are required to characterize fully this viral protease.

Whether NS2 itself plays a separate role in viral replication is yet to be determined. It has a very hydrophobic profile (Santolini *et al.* 1995) and is a transmembrane protein, with the C-terminus located in the ER lumen and the N-terminus in the cytoplasm (Dubuisson *et al.* 1994). It has been suggested that NS2 may play a role in virus assembly, as it has been co-precipitated with the envelope proteins, and the cleavage between E2-p7 and NS2 occurs after the complete folding of E2 (Dubuisson *et al.* 1994). Whether this function is correct remains to be determined, through analysis of NS2 in the context of productive viral replication, as opposed to *in vitro* expression systems.

1.2.2.7 The NS3 region

The NS3 region encodes protein with serine protease, NTPase, and RNA helicase activities. The serine protease activity has been particularly well characterized and probably plays an essential role in HCV processing, as a flavivirus homologue that has previously been shown to be indispensable for viral growth (Chambers *et al.* 1990), making it a desirable target for antiviral drugs. The activity resides in the N-terminal third of the NS3 protein and functions independently in two sets of processing events: (1) cleavage of the NS2/3 site, in conjunction with NS2 (Grakoui *et al.* 1993b, Hijikata *et al.* 1993b), and (2) release of the remaining downstream NS proteins, by mediating cleavage at the 3/4A, 4A/4B, 4B/5A, and 5A/5B sites (Bartenschlager *et al.* 1993, Grakoui *et al.* 1993b, Hijikata *et al.* 1993b, Tomei *et al.* 1993). The first 181 amino acids of NS3 (amino acids 1027–1207 of the polyprotein) have been shown to contain the domain necessary for all of these cleavages (Lin *et al.* 1994), with the minimal protease region narrowed down to amino acids 1059–1204 (Yamada *et al.* 1998). Amino acid sequence comparisons of several HCV isolates showed that the NS3 protein contains three highly conserved residues, His-1083, Asp-1107, and Ser-1165, which are known to represent the catalytic triad of the serine proteinase family (Chambers *et al.* 1990). Substitutions at any one of these residues abolish processing at the downstream sites (Bartenschlager *et al.* 1994, Grakoui *et al.* 1993c, Tomei *et al.* 1993). The cleavage between NS3/4A occurs in *cis* (Bartenschlager *et al.* 1993), and while those at the other sites can occur in *trans* (Bartenschlager *et al.* 1993, Lin *et al.* 1994, Tomei *et al.* 1993), the type of cleavage that dominates during viral replication has not been established.

Each of the NS3-dependent cleavage sites contains certain residues that are conserved among both sites and genotypes, suggesting their importance for substrate specificity (Hijikata *et al.* 1993b, Komoda *et al.* 1994). These residues include an Asp or Glu at P6, a Cys or Thr at P1, and a Ser or Ala at P1', although *in vitro* studies show that certain sites are more tolerant of substitutions than others (Bartenschlager *et al.* 1995b, Kolykhalov *et al.* 1994, Zhang *et al.* 1997). The cleavage site is distinct from that in the

classic flaviviruses, in which the NS3-dependent cleavages usually occur after dibasic amino acids (Chambers *et al.* 1990).

The NS4A protein functions as a cofactor in NS3 activity. It is essential for the NS3/4A and NS4B/5A cleavage events and enhances cleavage at the NS4A/4B and NS5A/5B sites (Failla *et al.* 1995, Lin *et al.* 1994, Tanji *et al.* 1995). The N-terminal 22 amino acids of NS3 have been shown to interact with NS4A (Alter *et al.* 1990, Failla *et al.* 1995, Tanji *et al.* 1995), creating a stable complex between the two proteins.

The crystal structure of the NS3 protease has been elucidated (Kim *et al.* 1996, Yan *et al.* 1998), and the solution structure of the N-terminal domain has been solved by nuclear magnetic reasonance (Barbato *et al.* 1999, Bartenschlager 1999). The protein has a structural zinc-binding site coordinated by residues Cys-1123, Cys-1125, Cys-1171, and His-1175 (Kim *et al.* 1996, Love *et al.* 1996, Stempniak *et al.* 1997), mutation of any of which reduces protease activity (Hijikata *et al.* 1993b, Stempniak *et al.* 1997). These analyses of the NS3 structure indicated that the substrate-binding pocket (SBP) is determined primarily by residues Leu-1161, Phe-1180, and Ala-1183, with Phe-1180 forming the bottom of the SBP. In substitutions at several sites around the SBP, only mutations of Phe-1180 substantially affected specificity (Koch and Bartenschlager 1997), confirming the importance of this residue in substrate recognition.

The RNA helicase (Hong *et al.* 1996, Kim *et al.* 1995, Tai *et al.* 1996) and NTPase (Hong *et al.* 1996, Suzich *et al.* 1993) activities of NS3 reside in the C-terminal 465 amino acids of the NS3 protein. These functions are less well characterized than the serine protease activity, although NS3 appears to possess properties similar to those of the helicases of other positive-strand RNA viruses and contains characteristic sequence motifs (Hong *et al.* 1996, Suzich *et al.* 1993). The helicase unwinds double-stranded RNA, double-stranded DNA, and RNA-DNA heteroduplexes in the 3' to 5' direction, using any NTP or dNTP as an energy source (Gwack *et al.* 1997, Tai *et al.* 1996). Biochemical characterizations (Porter 1998, Preugschat *et al.* 1996) and the X-ray crystallographic structure (Kim *et al.* 1998) of the helicase have recently been reported, confirming structural features common to other helicases. Mutations of certain residues within the conserved sequence motifs severely affect the enzymatic functions (Heilek and Peterson 1997, Kim *et al.* 1997, Lin and Kim 1999). These data, combined with biochemical and structural analyses, provide an understanding of the mechanism of action for the helicase and the basis for therapeutic drug design.

1.2.2.8 The NS4 region

The NS4 region encodes two viral proteins, designated NS4A and NS4B. The function of NS4B, a very hydrophobic protein, is unknown, although it is required for the phosphorylation of NS5A, together with NS4A and NS3 (Neddermann *et al.* 1999, Tanji *et al.* 1995). NS4A also has been shown to act as a cofactor in NS3 protease activity (see Section 1.1.2.7).

NS4A is 54 amino acids in length and has a hydrophobic N-terminal region followed by a hydrophilic C-terminus (Failla *et al.* 1994). NS4A enhances the NS3 serine protease cleavages in *cis* and in *trans* (Bartenschlager *et al.* 1994, Failla *et al.* 1994, Lin *et al.*

1994, Tanji *et al.* 1995). The precise region of NS4A essential for the activation of NS3 has been mapped to the central, hydrophobic portion of the protein encompassed by amino acids 21 to 34; a peptide spanning this region is sufficient to enhance cleavage by NS3 (Bartenschlager *et al.* 1995b, Failla *et al.* 1995, Lin *et al.* 1995, Shimizu *et al.* 1996b). A stable physical interaction occurs between the two proteins that can be destabilized by mutations in NS4A (Failla *et al.* 1995, Lin *et al.* 1995, 1997) and inhibited by a peptide carrying a single residue mutation at position 28 (in the central hydrophobic region) (Shimizu *et al.* 1996b). Although NS4A is not essential for all the processing functions of the serine protease, it appears to form an integral part of the protease complex (Kim *et al.* 1996), possibly stabilizing the protein in an active conformation and directing it to membranes where proteolytic processing and RNA replication occur (Failla *et al.* 1995, Kim *et al.* 1996, Lin *et al.* 1995). NS4A also has been shown to form a complex with the NS4B/NS5A polyprotein product (Lin *et al.* 1997), in which the NS4A central hydrophobic region is again essential. This interaction may explain the requirement for NS4A as a cofactor for cleavage by NS3 at this site.

1.2.2.9 The NS5 region

This region is processed into the proteins NS5A and NS5B. The primary function of NS5A is unknown, although from comparative studies with other viruses it is predicted to play a role in RNA replication. The mature NS5A exists as a phosphoprotein (p56); the phosphorylation occurs primarily on serine residues through the action of unknown cellular kinases (Reed and Rice 1997, 1999). Two phosphoproteins, p56 and p58 (phosphorylated and hyperphosphorylated forms, respectively), have been identified in HCV genotype 1b (Asabe *et al.* 1997, Duvet *et al.* 2002, Koch and Bartenschlager 1999, Neddermann *et al.* 1999), where the production of p58 requires NS3, NS4A, and NS4B expression in *cis* (Koch and Bartenschlager 1999, Neddermann *et al.* 1999). Two domains have been shown to be required for phosphorylation of NS5A (Asabe *et al.* 1997, Tanji *et al.* 1995), located around the center (amino acids 2200–2250) and C-terminus (amino acids 2350–2419). The major phosphorylation site for HCV-H (genotype 1a) has been mapped to Ser-2321 (Reed and Rice 1999), and mutation analysis has identified residues Ser-2197, Ser-2201, and Ser-2204 as putative hyperphosphorylation sites for p58 production by HCV-J (genotype 1b) (Tanji *et al.* 1995).

In addition to its potential involvement in RNA replication, clinical studies suggest that NS5A may play a role in conferring sensitivity to interferon-α through interaction with the interferon-induced cellular protein kinase R (Gale *et al.* 1999).

NS5B forms the C-terminal protein of the HCV polyprotein. It contains Gly-Asp-Asp (a GDD motif) at residues 2737–2739, which is common to other RNA-dependent RNA polymerases (Poch *et al.* 1989), and appears to be a membrane-associated phosphoprotein (Hong *et al.* 1996). RNA-dependent RNA polymerase activity has been demonstrated for recombinant NS5B expressed in insect cells (Behrens *et al.* 1996, Lohmann *et al.* 1997) and *Escherichia coli* (Ferrari *et al.* 1999, Oh *et al.* 1999), and RNA-binding domains have been identified within this region (Cheng *et al.* 1999, Lohmann *et al.* 1997). Four amino acid motifs have been shown to be essential for polymerase activity (Lohmann *et al.* 1997); designated A (amino acids 2640–2645), B (2702–2711), C (2737–2739), and D

(2762–2766), these appear to be important for nucleotide binding and catalysis (A), template/primer positioning (B), and NTP binding and catalysis (C and D).

Most of the recombinant NS5B proteins studied direct replication of RNA in a primer-dependent manner (Behrens *et al.* 1996, Lohmann *et al.* 1997). The primer may be an exogenous RNA molecule or may be generated through folding of the input RNA at the 3' end. However, a full-length form of NS5B expressed in *Escherichia coli* was able to synthesize genome-length HCV in a primer-independent manner (Oh *et al.* 1999). This protein also recognized specific sequences at the 3' end of positive- and negative-strand HCV RNAs for the initiation of RNA synthesis, suggesting that this sequence may more closely represent the authentic HCV RNA-dependent RNA polymerase (Oh *et al.* 1999). Replication of the HCV genome probably involves negative-strand RNA synthesis initiated by interaction between the NS5B polymerase or replicative complex and the 3' terminus of the viral genome. Specificity of binding by recombinant NS5B is not limited to RNA templates containing the 3' UTR of HCV (Behrens *et al.* 1996, Lohmann *et al.* 1997, Oh *et al.* 1999), although conserved stem-loop structures within the 3' coding region of the HCV RNA have been shown to specifically interact with NS5B (Cheng *et al.* 1999). The mechanism of HCV replication is yet to be determined, as are the other components of the replicative complex; analyses of the RNA-dependent RNA polymerase provide important contributions toward understanding this process and the possible development of antiviral agents targeting this protein.

1.2.3 Replication

One of the major hurdles in testing both neutralizing antibodies and antiviral drugs to combat HCV infection is the lack of an effective cell culture system for the virus. The data regarding the functions of HCV genes and replication of the virus have been derived from established mammalian cell expression systems or the chimpanzee animal model. The use of infectious RNA transcripts in chimpanzees has demonstrated the requirements for 3' UTR elements and functional enzymatic activities in HCV replication (Kolykhalov *et al.* 2000, Yanagi *et al.* 1999). However, this model is too expensive for extensive analyses of viral replication. Unfortunately, no tissue culture system has been developed that reliably supports HCV growth to the extent that experiments on replication can be conducted.

The genomes of positive-sense RNA viruses, such as flaviviruses or picornaviruses, act as both message molecules and as the templates for replication of the negative-strand RNA. The viral proteins involved in RNA replication must be translated for replication to occur. In the case of HCV, the structural proteins also must be translated, as they are coded at the 5' end of the viral RNA. However, experience with HCV subviral replicons suggests that the nonstructural proteins are not required for RNA replication (Blight *et al.* 2000, Lohmann *et al.* 1999). In addition, NS2 does not seem to be required. It is known that NS5B functions as the RNA-dependent RNA polymerase (see Section 1.2.2.9), the enzyme responsible for copying both the positive-strand RNA to a negative strand and for transcribing the negative strand back into the genomic RNA (Behrens *et al.* 1996, Lohmann *et al.* 1997). The NS3 protein is required to cleave the polyprotein into mature functional proteins. In addition, the carboxy-terminal portion of NS3 has helicase activity, which presumably is important for unwinding the positive and negative strands

of RNA. The exact functions of NS5A and NS4 in the replication process have not been determined. However, all the nonstructural proteins, NS3 through NS5B, have been identified in a membranous replication complex (Gosert *et al.* 2002).

1.3 Host range and target cells

1.3.1 Tissue culture

There have been several reports of mammalian cells able to support the growth of HCV. The cell lines used include MT2 (human T-lymphoctes infected with human T-cell leukemia virus [HTLV]), peripheral blood mononuclear cells, other lymphocyte cell lines, and hepatocytes from humans, chimpanzees, and tree shrews (Major and Feinstone 1997). Enhancement of HCV replication in MT2 cells by Epstein-Barr virus (EBV) was recently reported; in particular, the gene *EBNA1* was thought to be involved (Sugawara *et al.* 1999). These systems rely on the use of reverse transcriptase-PCR (RT-PCR) for detection of the virus and, in particular, on detection of the negative strands of HCV RNA by strand-specific RT-PCR as evidence of viral replication. Data indicating the presence of negative-strand RNA should be interpreted with care, as specificity and sensitivity can vary depending upon the choice of primers and reaction conditions (Lanford *et al.* 1994, Leary *et al.* 1996). Although HCV has been convincingly demonstrated to replicate in a number of cell lines, the low level of replication has limited the utility of such *in vitro* systems. With further development, some of these systems may provide a robust *in vitro* culture system for HCV that is useful for basic virology, as well as viral production and immunology studies.

For example, a recent study demonstrated that tree shrew (*Tupaia belangeri*) hepatocytes may be used as a model to study HCV infection (Zhao *et al*. 2002). Hepatocytes incubated with HCV serum exhibited time-dependent HCV RNA synthesis. The HCV RNA also was detected in the culture medium and was shown to be resistant to nuclease, indicating that the newly synthesized HCV RNA was packaged into viral particles. Further, the study indicated formation of new viral genomes during infection, *de novo* synthesis of HCV proteins in the infected tree shrew hepatocytes, and passage of HCV from infected to naïve hepatocytes. Also, putative viral structures were observed in infected tree shrew hepatocytes by electron microscopy.

Alternative approaches to studying virus assembly or the role of viral antigens in replication have involved the production of virus-like particles (Baumert *et al.* 1998) or pseudotype vesicular stomatitis virus particles containing E1 or E2 (Lagging *et al.* 1998). These systems provide a potential means of studying envelope glycoprotein interactions, elements essential for particle assembly, and neutralization of antibody activity.

Although complete viral replication *in vitro* has not been achieved, a subgenomic replicon containing most of the nonstructural proteins of HCV recently was shown to replicate in the hepatoma cell line HuH7 (Lohmann *et al.* 1999). In this replicon system, viral RNA and proteins could be radiolabelled. Very high levels of replication have been achieved by selection of mutants of these subgenomic replicons. Such systems may provide the basis for defining functional HCV replication units and testing antiviral drugs.

The basic replication strategy of HCV is expected to be similar to that of other flaviviruses. The virus binds to a cell-surface receptor, is taken into the cell, and is uncoated. The viral genome serves as both message and the template for RNA negative-strand replication. A double-stranded replicative intermediate may exist briefly, but the two strands are unwound by the viral helicase, and each strand may serve as the template for additional rounds of replication, with the positive strand also being used as the message molecule for translation of the viral polyprotein and being encapsidated as the viral genome.

Although the details of this replication scheme are not well characterized, some features of HCV replication have been studied. Currently, three candidate cellular receptors exist for HCV. The tetraspanin molecule CD81 has been shown convincingly to bind to the E2 glycoprotein (Patel *et al.* 2000, Pileri *et al.* 1998). However, there is little evidence that this binding initiates a productive infection. The receptor for LDL or VLDL also has been proposed as the receptor for HCV (Agnello *et al.* 1998). It seems clear that the virus is complexed with LDL or VLDL and that it therefore may bind to the LDL receptor on cells. Data indicate that this binding is associated with virus entry, but there is no evidence that it initiates infection. Recently, a member of the scavenger receptor family has been shown to bind to HCV and to have some features of a receptor (Cerino *et al.* 2002). Again, however, data indicating that this molecule functions as the true HCV receptor are limited.

It is known that replication occurs in association with cellular membranes, and a membranous web-like structure has been identified that includes the viral proteins associated with replication (Gosert *et al.* 2002). It is believed that the virus particle itself buds off internal membranes, probably into the lumen of the ER, and the particles then transit through the Golgi complex and out of the cell.

1.3.2 Animal models

One of the major obstacles facing HCV investigators is the lack of a small-animal model for HCV infection and disease. The problem is compounded by the fact that *in vitro* culture systems also are inadequately robust for most studies. At present, the chimpanzee is the only established animal model that both is permissive for HCV infection and develops a disease syndrome mimicking that seen in humans. Although an exhaustive search among other nonhuman primates has not been made, studies in most Old World and New World primates have not yielded positive results, despite scattered, unconfirmed reports of infections in tamarins and macaques.

Because of the restrictions on doing research in chimpanzees, alternative *in vivo* models have been developed. Various transgenic mouse systems have been produced in which HCV proteins are synthesized and which have been useful for studying the pathogenensis of HCV (Koike *et al.* 2002). Several of these models are described in Section 1.3.2.3. Further development of transgenic systems will eventually provide tools useful for HCV research and partially alleviate the need for chimpanzees. For further discussion, see Section 4.

1.3.2.1 Chimpanzee

The chimpanzee (*Pan troglodytes*) is genetically more than 98.5% identical to man. It has been possible to infect chimpanzees with all of the established human hepatitis viruses that have a limited host range and for which *in vitro* culture systems are not readily available. All of these hepatitis viruses can induce a disease in chimpanzees similar to that in humans. In general, viral hepatitis in chimpanzees is milder than the same viral infection in humans. At present, the chimpanzee is the only proven animal model for HCV infection and disease (Lanford and Bigger 2002). Chimpanzee studies provided the first evidence for HCV transmission (Hollinger *et al.* 1978), a means of measuring infectivity, and material for cloning and characterizing the HCV genome (Choo *et al.* 1989). Evidence for a lack of protective immunity against HCV, evidence for the role of HVR1, and insights into possible vaccine strategies also have been gained from chimpanzee studies. Finally, this model has provided the only reliable means of testing the infectivity of transcribed RNAs from molecularly cloned putative full-length HCV cDNA (Kolykhalov *et al.* 1994, Yanagi *et al.* 1997).

In most aspects, the virologic and clinical presentation of acute HCV infection is similar in humans and chimpanzees. Similarities in presentation include onset of disease, level of viremia, and timing of serologic response and liver damage, as indicated by alanine aminotransferase (ALT) elevation. However, some differences exist, and only approximately 30% to 50% of chimpanzees develop persistent HCV infections (Bassett *et al.* 1998), compared with as many as 70% to 80% of humans.

The onset of hepatitis C in chimpanzees typically is mild. Although approximately 25% of human patients with transfusion-associated hepatitis C become jaundiced, there are no reports of jaundice in a chimpanzee due to HCV infection. The milder nature of the disease in chimpanzees is also reflected in lesser degrees of hepatic injury measured by ALT levels or histopathologic changes. Although both chronic hepatitis and hepatocellular carcinoma have been reported, chimpanzees rarely develop significant chronic liver disease (Walker 1997). Observations of a low incidence of chronic disease may be biased, however, by the young age at which many animals are infected and the short observation period.

Humoral immune responses to HCV structural proteins are observed less frequently and are significantly weaker in chimpanzees than in humans (Akatsuka *et al.* 1993, Cerino and Mondelli 1991, Choo *et al.* 1994). However, even in the absence of strong humoral immune responses, the cellular immune response has been described as quite vigorous in chimpanzees with acute, self-limited HCV infection (Cooper *et al.* 1999). Termination of HCV infection correlates precisely with the onset of a multispecific intrahepatic cytotoxic T-lymphocyte (CTL) response, which can persist for more than one year after resolution of the infection (Cooper *et al.* 1999).

The development of infectious RNA transcripts from a functional molecular clone allowed infection and pathogenesis studies with a single well-defined viral polyprotein sequence. This development greatly simplified the study of HCV evolution during acute and chronic infection and the analysis of HCV-specific immune responses (He *et al.* 1999, Kolykhalov *et al.* 1997, Yanagi *et al.* 1997).

After intrahepatic injection of transcribed full-length uncapped RNAs, circulating HCV RNA generally could be detected in the serum by one week post-inoculation and increased during the first 10 to 12 weeks (Kolykhalov *et al.* 1997). ALT peaked at weeks 10 to 14 after infection, and in most animals, seroconversion was observed after 2 to 3 months (Kolykhalov *et al.* 1997). The severity of intrahepatic histologic changes, especially portal inflammation and focal necrosis, was typical for hepatitis C in chimpanzees and paralleled ALT elevations. These pathogenic profiles are strikingly reminiscent of those obtained in chimpanzees inoculated with infectious HCV-containing samples. These experiments proved that circulating RNA is indeed due to authentic viral replication, rather than release of inoculated nucleic acid, and defined the elements of a functional HCV genome. In addition, the demonstration that RNA transcribed from cloned HCV cDNA could initiate infection and cause hepatitis in chimpanzees also provided the formal proof that HCV alone is the causative agent of this disease.

1.3.2.2 Tree shrews

Chinese tree shrews (*Tupaia belangeri chinensis)* caught in the wild in Yunnan, China, have been shown to be susceptible to HCV infection. These animals have been experimentally infected with HCV genotype 1b in China (Study A) and a mixture of genotypes 1a, 1b, and 3 in Spain (Study B) (Xie *et al.* 1998). Study A included two control animals and 23 inoculated animals. Animals in study B were divided into three groups: Group I consisted of 10 animals inoculated with HCV, group II of 4 animals exposed to whole-body irradiation with 750 cGy X-rays and then inoculated with HCV, and Group III of 4 control animals.

Eight animals (35%) in Study A developed HCV viremia, compared with 20% and 50% in Groups I and II, respectively, in Study B. Viremia was transient or intermittent, with a low rate of anti-HCV seroconversion. In addition, anti-HCV antibodies were detected transiently in the sera of 7 animals in study A, but persisted in 3 animals for one to two months. Transaminases peaked in most cases between weeks 7 and 18 in Study A and between weeks 8 and 12 in Study B. In Study A, liver biopsies were obtained 50 weeks postinoculation from 4 animals with previous elevation of ALT and/or detectable anti-HCV antibody. Liver pathology included degeneration of hepatocytes, multinucleated liver cells, and macrovesicular focal necrosis with inflammatory infiltrate. Similar liver pathology was observed in all infected animals and in 3 control animals in Study B after 27 weeks. Liver biopsies were repeated at week 33 in 2 animals from Group II and in 2 controls. Electron microscopic examination revealed fat droplets, hyperplasia of smooth endoplasmic reticulum, and multiple membranous arrays resembling myelin figures in inoculated animals but not in controls. In Study B, higher titers of anti-HCV antibodies and longer-lasting viremia occurred in animals preexposed to X-rays. The authors were unable to attribute any of the pathological changes in the liver solely to HCV infection because of the wild nature of the animals.

1.3.2.3 Mice

Although HCV does not infect mice, several mouse models have proven valuable in hepatitis C research. Mice that are transgenic for human major histocompatibility complex (MHC) molecules, for example, have been used efficiently to identify HCV

epitopes and to test vaccination strategies. Multiple human leukocyte antigen (HLA)-A2 restricted T-cell epitopes of the hepatitis C virus have been defined by immunization of mice with peptides, DNA vaccines, or vaccinia viruses expressing HCV sequences (Oseroff *et al.* 1998, Shirai *et al.* 1995, Wentworth *et al.* 1996). These epitopes were processed endogenously and also were recognized by HCV-infected humans. Thus, mice transgenic for human MHC molecules are an excellent model system in which to develop immunotherapeutic vaccines that may be used in humans. For example, a multi-epitope vaccine has been shown to induce simultaneous CTL responses to a pool of five different epitopes (Oseroff *et al.* 1998).

Transgenic mice that produce individual or several HCV proteins have been used to analyze the biosynthesis of HCV proteins and their pathogenic role. Liver-specific production of HCV core and E2 proteins resulted in the predominantly cytoplasmic presence of core protein, with occasional nuclear staining, and presence of the E2 protein in both cytoplasm and membranes. Importantly, the livers of two independent mouse lineages remained histologically normal for the entire observation period (i.e., until the mice were 6 and 18 months of age) (Kawamura *et al.* 1997, Pasquinelli *et al.* 1997). In contrast, transgenic mice producing HCV core protein under the control of a regulatory region from the hepatitis B virus developed progressive hepatic steatosis (Moriya *et al.* 1997), and one-fourth of the male transgenic mice developed hepatocellular carcinoma (Moriya *et al.* 1998). This effect was specific for the expression of HCV core as a transgene, since transgenic mice that carried HCV E1 and E2 under the same transcription control region did not have any neoplastic liver lesions (Koike *et al.* 1995).

Finally, immunodeficient mice have been used to test the hypothesis that HCV persists in peripheral blood mononuclear cells (PBMC). Specifically, severe combined immunodeficiency (SCID) mice have been intraperitoneally inoculated with hematopoietic cells from HCV-infected subjects (Bronowicki *et al.* 1998), and HCV-infected human liver fragments have been transplanted under the kidney capsule of immunodeficient BNX mice (Galun *et al.* 1995). The studies suggest that HCV persists in mononuclear cells at a low rate of multiplication.

In another model, transgenic mice that expressed the urokinase plasminogen activator (uPA) gene under an albumin promoter were crossed with SCID mice. Urokinase over-production in the liver accelerates hepatocyte death (Mercer *et al.* 2001). It was found that adult human hepatocytes could repopulate the livers of these mice, presumably under the stimulation of the liver regeneration factors liberated by the transgenic mouse. It was then demonstrated that the human hepatocytes of the chimeric immunodeficient mice that were homozygous for the uPA transgene could be infected with HCV and sustain persistent infections. Although this system is not easy to establish or maintain, it does seem to offer the possibility of performing certain HCV infectivity experiments without chimpanzees. This model also may be useful for testing antiviral agents in an *in vivo* persistent infection system.

1.4 Summary

HCV causes most parenterally transmitted non-B viral hepatitis. HCV was originally identified in a persistently infected chimpanzee through the use of serum from non-A,

non-B heptatis patients as a source of specific antibody. It was shown to be a positive sense single stranded RNA virus of the Flaviviridae family. The Flaviviridae comprises three distinct genera: the classic flaviviruses, the pestiviruses of animals, and the hepaciviruses, of which hepatitis C is the sole example.

The HCV viral particle is approximately 50 nm in diameter; enveloped, virus-like particles have been identified via electron microscopy. The viral particle consists of an envelope derived from host membranes, into which are inserted the virally encoded glycoproteins (E1 and E2), surrounding a nucleocapsid and a positive-sense single-stranded RNA genome of approximately 9,600 nucleotides. The HCV genome contains highly conserved UTRs at both the 5' and 3' termini, which flank a single ORF encoding a polyprotein of approximately 3,000 amino acids. The 5' UTR has been shown to direct translation, and the 3' UTR probably is involved in viral replication and possibly translation. The polyprotein is processed co- and post-translationally by cellular and viral proteases to produce specific viral gene products. The structural proteins (core, E1, and E2) are located in the N-terminal quarter, and the nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) in the remaining portion of the polyprotein.

The first 191 amino acids of the HCV polyprotein constitute the putative nucleocapsid, or core protein. The RNA binding activity has been localized to the N-terminus, amino acids 1–75; this sequence is characterized by a cluster of basic residues showing a high degree of conservation, suggesting an important biological function. The core protein has been shown to interact *in vitro* with several biologically active cellular proteins, such as TNF-α and NF-κB, performing multiple functions in cells, including transactivation and suppression. The glycoproteins E1 and E2 (the putative viral envelope proteins) are targeted to the ER) by amino-terminal signal peptides and are N-terminally glycosylated. An unusually high degree of amino acid variation has been observed in the N-terminus of the E2 protein, a hypervariable region referred to as the HVR1. Some evidence suggests that this region is subject to immune pressure, with the potential for escape mutants. These data could provide important considerations for prophylactic vaccine development.

The nonstructural protein NS2 has a very hydrophobic profile and may play a role in virus assembly. The NS3 region encodes protein with serine protease, nucleotide triphosphatase, and RNA helicase activities. The NS4 region encodes two viral proteins designated NS4A and NS4B. The NS4A protein functions as a cofactor in NS3 activity. It is essential for the NS3/4A and NS4B/5A cleavage events and enhances cleavage at the NS4A/4B and NS5A/5B sites. The function of NS4B, a very hydrophobic protein, is unknown, although it is required for the phosphorylation of NS5A, together with NS4A and NS3. The NS5 region is processed into the proteins NS5A and NS5B. The primary function of NS5A is unknown, but it is predicted to play a role in RNA replication and may play a role in conferring sensitivity to interferon-α.

HCV strains show extensive genome heterogeneity, although this variability is not uniformly distributed over the genome. Relatively well conserved regions of the HCV genome (core, E1, and NS5) have been studied extensively and used as the basis for genotype classification. All currently known isolates of HCV can be divided into six phylogenetically distinct groups designated as clades 1 to 6 (consisting of the previously

identified genotypes). All types of HCV isolated to date have the potential to cause serious chronic liver disease, although infection with some types may be more responsive to treatment with interferon.

One of the major hurdles in testing both neutralizing antibodies and antiviral drugs to combat HCV infection is the lack of an effective cell culture system for the virus. The data regarding the functions of HCV genes and replication of the virus have been derived from established mammalian cell expression systems or the chimpanzee animal model. HCV replication is believed to occur entirely within the cytoplasm and is closely associated with cytoplasmic membranes. The basic replication strategy of HCV is expected to be similar to that of other flaviviruses. The virus binds to a cell-surface receptor, is taken into the cell, and is uncoated. Currently, three candidate cellular receptors exist for HCV: the tetraspanin molecule CD81, the receptor for LDL or VLDL, and a member of the scavenger receptor family. The viral genome serves as both message and the template for RNA negative-strand replication. A double-stranded replicative intermediate may exist briefly, but the two strands are unwound by the viral helicase, and each strand may serve as the template for additional rounds of replication, with the positive strand also being used as the message molecule for translation of the viral polyprotein and being encapsidated as the viral genome. Replication occurs in association with cellular membranes, and it is believed that the virus particle itself buds off internal membranes, probably into the lumen of the ER, and the particles then transit through the Golgi complex and out of the cell.

At present, the chimpanzee is the only established animal model that both is permissive for HCV infection and develops a disease syndrome mimicking that seen in humans. In general, viral hepatitis in chimpanzees is milder than the same viral infection in humans, and chimpanzees rarely develop significant chronic liver disease, although both chronic hepatitis and hepatocellular carcinoma have been reported. Humoral immune responses to HCV structural proteins are observed less frequently and are significantly weaker in chimpanzees than in humans; however, the cellular immune response has been described as quite vigorous in chimpanzees with acute, self-limited HCV infection. Tree shrews have been shown to be susceptible to HCV infection. These animals have been experimentally infected with HCV genotypes 1a, 1b, and 3, but viremia was only transient or intermittent, with a low rate of anti-HCV seroconversion.

Various transgenic mouse systems have been developed in which HCV proteins are produced; these models have been useful for studying the pathogenesis of HCV. Mice that are transgenic for human MHC molecules, for example, have been used efficiently to identify HCV epitopes and to test vaccination strategies. Transgenic mice that produce one or several HCV proteins have been used to analyze the biosynthesis of HCV proteins and their pathogenic role. Human hepatocytes have been grown in immunodeficient mice *in vivo* after destruction of the mouse liver by overexpression of the urokinase plasminogen activator gene. These human hepatocytes could be infected by HCV and sustain persistent infections.

2 Human Exposure

2.1 Introduction

Approximately 3 to 4 million people in the United States are infected with HCV (Alter *et al.* 1999). The major route of transmission is through contaminated blood, and the major risk factor is intravenous drug use. HCV is the leading cause of known liver disease in the United States. This section describes methods for detecting HCV; the epidemiology of HCV infection, including prevalence, incidence, transmission, and risk factors; the natural history of HCV infection, including clearance and progression to clinical disease other than cancer; and treatment of HCV.

2.2 Methods of detection

Assays have been developed for diagnostic detection of HCV infections in individuals, and some of these methods have been adapted for detection of HCV contamination of blood or blood products. The tests used to detect HCV in individuals are discussed below. Two general categories of diagnostic tests for HCV exist: (1) serological assays that detect circulating antibodies against the virus and (2) HCV RNA detection assays. The latter category of molecular assays can be divided into those that detect, quantify, or characterize the HCV RNA genome. The discussion below is not intended to list exhaustively all commercially available assays for HCV serology or RNA. Assays listed by name and manufacturer are limited to those that have received U.S. Food and Drug Administration (FDA) approval; other assays not listed here also may be used for diagnostic purposes by physicians or for research purposes in epidemiologic studies.

The FDA currently approves three assays for HCV-encoded antigen: (1) Abbott HCV Enzyme Immunoassay (EIA) 2.0, (2) Ortho HCV Version 3.0 Enzyme-Linked Immunosorbent Assay (ELISA) Test System, and (3) Chiron Recombinant Immunoblot Assay (RIBA) HCV 3.0 Strip Immunoblot Assay (SIA) (FDA 2002) (see Table 2-1). In addition, the FDA has approved one assay for HCV RNA, the Amplicor Hepatitis C Virus Test from Roche Molecular Systems, Inc. (Pleasanton, CA). Other assays, such as the branched DNA assay and transcription-mediated amplification assay, also are available commercially. The methodologies on which the various assay types are based are described in Table 2-2.

Table 2-1. FDA-approved and -licensed tests for antibodies against hepatitis C virus antigen (anti-HCV assays)

Trade name	Format	Sample	Use	Manufacturer	Approval date
Abbott HCV EIA 2.0	EIA	Serum or plasma	Donor screen	Abbott Laboratories Abbott Park, IL U.S. License 0043	5/6/1992
Ortho HCV Version 3.0 ELISA Test System	EIA	Serum or plasma	Donor screen	Ortho-Clinical Diagnostics, Inc. Raritan, NJ U.S. License 1236	5/20/1996
Chiron RIBA HCV 3.0 Strip Immunoblot Assay	SIA	Serum or plasma	Donor supplemental	Chiron Corporation Emeryville, CA U.S. License 1106	2/11/1999

Source: FDA 2002.

Table 2-2. HCV assay methodologies

Assay	Description
Serological Assays	
EIA or ELISA	Based on capture of anti-HCV molecules by antigen molecules immobilized on a solid surface. The captured anti-HCV molecule is then detected by a labeled antibody to human immunoglobulin (Ig).
SIA or RIBA	Often used to confirm EIA results. The recombinant c100-3 peptide is immobilized as a band on a test strip and exposed to serum. If antibodies specific to HCV are present, they will bind to the immobilized antigen molecule, and the antigen-antibody complex can be visualized by addition of peroxidase-labeled anti-human IgG conjugate followed by development of the colorimetric reaction with peroxidase substrate.
Qualitative RNA Assay	
Transcription-mediated amplification	Based on amplification of HCV RNA in the presence of an internal control RNA. Moloney murine leukemia virus (MMLV) reverse transcriptase creates a cDNA for HCV RNA incorporating a promoter sequence for T7 polymerase. T7 polymerase then catalyzes transcription of multiple copies of the RNA, which are detected by chemiluminescent-labeled single-stranded nucleic acid probes.
Quantitative RNA Assays	
Amplicor Hepatitis C Virus Test	Uses RT-PCR amplification of a 244-nucleotide segment of the 5' UTR of the HCV genome to detect HCV RNA. The biotin-labeled products are captured by oligonucleotide probes bound to magnetic particles and detected by a colorimetric reaction. A synthetic RNA sequence is added to samples as a positive internal control for extraction of nucleic acids. Possible contamination from previous amplifications is minimized by addition of AmpErase, a uracil DNA glycosylase that eliminates previously amplified DNA molecules.

Assay	Description
Branched DNA assay	Uses a nucleic acid probe to quantify directly HCV RNA in serum or plasma. A third-generation branched DNA assay uses five basic components to provide quantification of HCV RNA: a capture probe attached to a microwell plate, a capture extender molecule, a label extender, a preamplifier molecule, and a set of amplifier molecules (alkaline phosphatase). The label extender, preamplifier molecule, and amplifier molecules contain synthetic nucleotides to minimize nonspecific probe interactions. Amplification of the signal is obtained by binding of multiple alkaline phosphatase molecules to each HCV RNA molecule. In one assay from the Chiron Corporation (Emeryville, CA), 18 branched DNA molecules can bind to each HCV RNA molecule, each branched DNA molecule has 15 arms, and each arm can hybridize to three alkaline phosphatase molecules.

2.2.1 Serological assays

Cloning of the HCV genome and production of the recombinant antigen c100-3 in yeast made detection of antibodies against HCV possible. Two types of immunoassays, EIA and SIA, were developed based on the c100-3 antigen, which is derived from the NS4 region of the viral genome. These immunoassays based on a single antigenic epitope are now referred to as first-generation HCV-antibody tests. Incorporation of additional epitopes has improved the specificity and sensitivity of the assays; these newer assays are referred to as second- and third-generation serologic assays. The EIA and SIA methods have developed in parallel, as discussed below.

Although EIAs can detect HCV antibodies in over 97% of infected individuals, the assays cannot distinguish among acute, chronic, and resolved infections (Larson and Carithers 2001). False-positive results do not occur frequently in the serologic assays, but they are most likely to involve patients with a low risk for infection with HCV (e.g., volunteer blood donors). On the other hand, false-negative results are likely to be obtained from patients who are immunosuppressed or who are receiving chronic dialysis. Historically, SIAs have been used to identify false-positive results; however, in patients who are suspected of having chronic HCV infection, tests for HCV RNA are more useful.

2.2.1.1 First-generation serologic assays

The HCV EIA was approved by the FDA and became commercially available in 1990. This assay also is referred to as an ELISA (IARC 1994). Although this first-generation assay reacted with 80% to 90% of sera from patients with chronic post-transfusion non-A, non-B hepatitis, a high rate of false-positive results also was observed. Higher specificity was obtained by incorporating the c100-3 antigen into an SIA or RIBA that could serve as a confirmatory test for HCV. Although the first-generation assays were important advances in detection of HCV infection, they had poorer specificity and sensitivity than the methods in use today (Abdel-Hamid *et al.* 2002).

Although the second- and third-generation assays have replaced the first-generation serologic assays, a few studies discussed in Section 3 were conducted with the first-generation assays.

2.2.1.2 Second-generation serologic assays

Improvements in the sensitivity and specificity of the second- and third-generation assays have been based on identification of additional antigenic sites within the HCV genome. The second-generation EIA and SIA incorporate the c100-3 antigen and the 5-1-1 antigen contained within it, the c22-3 antigen contained within the core region, and the c33c antigen from the NS3 nonstructural region (Germer and Zein 2001). These assays were approved by the FDA in 1992 (EIA) and 1993 (SIA). As with the first-generation assays, the second-generation SIA is used primarily to validate positive results, particularly those obtained from individuals considered to be at low risk for HCV infection (e.g., organ, tissue, and blood donors).

In a recent comparison with the third-generation EIA, with validation by a third-generation SIA, the second-generation EIA had a sensitivity of 89.9% and a specificity of 98.9% (Abdel-Hamid *et al.* 2002). Negative results must always be interpreted with caution because of the time, as much as 12 weeks, required for seroconversion to develop after exposure to the virus. Germer and Zein (2001) reported that indeterminate and false-negative results in the second-generation assays were most likely to be obtained with sera from immunocompromised and immunosuppressed patients, as well as those with acute infections.

2.2.1.3 Third-generation serologic assays

The FDA approved third-generation EIA and SIA serological assays in 1996 and 1999, respectively. These assays incorporate two recombinant antigens (c33c and NS5) and three synthetic peptides (c110p, 5-1-1p, and c22p). The NS5 antigen is a fifth antigen in addition to the four used in second-generation assays. Abdel-Hamid *et al.* (2002) reported a sensitivity of 99% and a specificity of 99.8% for the third-generation EIA.

Colin *et al.* (2001) assessed the sensitivity and specificity of third-generation serological HCV diagnostic tests by analyzing 10 studies selected from 132 identified by searches of the literature. The estimated sensitivity of the third-generation EIA was 98.9% (95% confidence interval [CI] = 94% to 100%) for patients with chronic liver disease, and the specificity was 100%. The estimated sensitivity with panels of serum was 97.2% (95% CI = 92% to 99%). The estimated sensitivity of the third-generation SIA in hemodialyzed patients was 78.8% (95% CI = 65% to 89%). Colin *et al.* (2001) reported that the third-generation EIAs have good sensitivity and specificity for high-risk patient groups; however, the authors stated that studies were not sufficient to assess the sensitivity and specificity of the assays in the general population. They noted that a perfect "gold standard" for assessment of HCV diagnostic assays has not yet been established.

The National Institutes of Health (NIH) Consensus Statement on management of HCV (NIH 2002) concluded that the very high sensitivity and specificity of the third-generation EIA obviated the need for RIBA to confirm diagnosis of individual patients with risk factors for HCV. The EIA and RIBA use the same antigens and are considered to have equivalent sensitivity. In immune-competent patients, a negative EIA test is considered sufficient to exclude a diagnosis of chronic HCV infection. False-negative results in the EIAs occur among patients on dialysis and those with immune deficiencies.

Autoimmune disorders may be a factor in false-positive EIA results. Although RIBA is no longer considered essential for routine diagnosis, it still may be useful in some research applications and as a supplemental assay in large-scale screening of blood products for HCV.

2.2.2 Molecular HCV assays

Molecular assays for HCV include qualitative and quantitative nucleic acid amplification tests, branched DNA tests, and HCV genotyping (Podzorski 2002). As discussed above, the serologic assays may give false-negative results in recently infected individuals or those who cannot produce an effective immune response. The RNA for HCV may be detected in these individuals by sensitive qualitative molecular assays. Quantitative RNA assays are based on the use of RT-PCR with an internal RNA standard, signal-amplification by branched DNA assay, or transcription-mediated amplification.

2.2.2.1 Qualitative HCV RNA assays

Qualitative HCV nucleic acid amplification tests are available as commercial tests from several companies, and some clinical laboratories have developed in-house assays (Podzorski 2002). These qualitative assays are not used to screen for HCV infection, but they may be used as confirmatory tests following positive second- or third-generation EIA tests. The basis of these qualitative HCV RNA assays is reverse transcription of the viral RNA to a complementary DNA (cDNA) and amplification by PCR. The RT-PCR assays are extremely sensitive, but they require careful quality control to eliminate contamination that may cause false-positive results. Another qualitative HCV RNA assay is based on transcription-mediated amplification; HCV RNA is amplified in the presence of an internal control RNA (Krajden *et al.* 2002, Linnen *et al.* 2002).

2.2.2.2 Quantitative HCV RNA assays

HCV viral load is often determined to predict the response to antiviral therapy (Podzorski 2002). Quantification of HCV viral load is a direct measurement of viremia and can provide information on viral kinetics. Determining the level of virus in infected individuals may be useful, as patients with lower viral load have been reported to have a more sustained response to treatment with interferon-α. Three quantitative procedures for HCV viral load testing are commercially available: (1) RT-PCR-based amplification assays, (2) branched DNA signal amplification assays, and (3) nucleic acid sequence-based amplification assays.

Selection of a quantitative assay method may depend on the type of data required. Lunel *et al.* (1999) comparatively evaluated HCV RNA quantitation by commercial assay kits based on each of the three methods. They reported that the amplification methods (RT-PCR and sequence-based amplification) were more sensitive than the branched DNA method for quantification of HCV RNA in patients with chronic HCV infection; however, the branched DNA and sequence-based amplification methods were more likely to quantify infection with all HCV genotypes.

RT-PCR-based HCV RNA assay

The Amplicor version 2.0 (Roche Molecular Systems; Belleville, NJ) manual assay, an RT-PCR-based HCV RNA assay, was approved by the FDA in 2001. A semi-automated version of the assay also is available, which uses a biotin-labeled primer to amplify the target sequence within the HCV RNA genome (Doglio *et al.* 1999). Nolte *et al.* (2001) concluded that both the manual and semi-automated Amplicor HCV tests had good overall agreement with serology results and with other markers of liver disease.

Branched DNA-based signal amplification assay

The branched DNA-based signal amplification assay uses a nucleic acid probe to directly quantify HCV RNA in serum or plasma (Ross *et al.* 2002). In one version of the assay, 18 branched DNA molecules can bind to each HCV RNA molecule, each branched DNA molecule has 15 arms, and each arm can hybridize to three alkaline phosphatase molecules (Wilber 1997), thus amplifying the signal to increase the assay's sensitivity.

Nucleic acid sequence-based amplification of HCV RNA

A nucleic acid sequence-based amplification system is based on the same principles as transcription-mediated amplification (see Section 2.2.2.1); however, addition of competitor RNA molecules at known concentrations allows quantitative determination of HCV RNA (Lunel *et al.* 1999). The RNA concentration is calculated from a standard curve based on the competitor RNA molecules.

2.2.2.3 HCV genotyping

Genotype determination could be a factor in determining clinical treatment for HCV infection, because some genotypes, particularly type 1b, are associated with increased severity of liver disease and a lower rate of response to interferon therapy than other genotypes (Conry-Cantilena 1997). The standard method for genotyping requires direct sequencing of the entire HCV genome obtained from a patient, but this method is not practical on a large scale (Germer and Zein 2001). Determining the sequence of a smaller portion of the HCV genome, specifically the NS5, core, or 5' UTR, can replace direct sequencing of the complete genome; however, even this more limited procedure is too complex to apply routinely to patient samples. Alternative methods that have been applied to clinical specimens are reamplification of RT-PCR-amplified RNA with type-specific primers, hybridization with type-specific probes, and RFLP analysis of amplification products. Some evidence exists for differences among HCV genotypes in geographic distribution, disease outcome, and response to therapy; for example, current recommendations for treatment of HCV include 48 weeks of therapy with interferon plus ribavirin for patients with genotype 1 infection, compared with 24 weeks of treatment for those infected with genotype 2 or 3 (McHutchison *et al.* 2002).

2.2.3 Detection of HCV in blood banks

Gresens and Holland (2001) reviewed the progressive steps taken by blood banks in the United States to reduce the risk of transfusion transmission of HCV. Beginning in 1987, the American Association of Blood Banks instituted a requirement for use of surrogate markers for non-A, non-B hepatitis (i.e., HCV); elevated levels of alanine aminotransferase and antibody to the hepatitis B core antigen (anti-HBc) were used for

this screening. This action resulted in almost no improvement in the rate of post-transfusion HCV seroconversion. Three years later, in 1990, the first-generation anti-HCV EIA was introduced for testing in United States blood banks. The use of this test resulted in an 80% reduction in the rate of transmission of HCV via transfusion. This reduction carried with it a cost in exclusion of donations as a result of a high rate of false-positive test results. Further improvements introduced in the second- and third-generation EIAs reduced the rates of both false-negative and false-positive results. The false-positive rate has been further reduced by use of a second, more specific assay, the RIBA. The blood supply in the United States also undergoes a third test, a nucleic acid test for HCV-RNA. Approximately 93% of the United States blood supply was tested by this method in 1999. Institution of these test methods for donated blood units has reportedly reduced the risk of transmission of HCV from 1 per 200 units to less than 1 per 1,000,000 units (CDC 2002). Risk is likely to be further reduced by application of methods currently under development to photochemically inactivate any potentially infectious organisms in blood or blood products.

2.2.4 Liver function and liver enzyme assays

Liver enzyme tests and other liver-related clinical assays are used to monitor liver function during acute or chronic hepatitis (Larson and Carithers 2001). Serum ALT, gamma glutamyltransferase, and alkaline phosphatase are the primary liver enzymes measured. Other liver-related clinical assays include serum bilirubin, albumin, and prothrombin time. A committee of the National Academy of Clinical Biochemistry has recommended guidelines for serum tests to be used to evaluate patients with either known or suspected liver disease, including hepatitis due to HCV infection. The test panel includes the following: aspartate aminotransferase, ALT, alkaline phosphatase, total bilirubin, direct bilirubin, total protein, and albumin. These assays, particularly the ALT assay, provide simple biochemical tests to assess liver disease activity and to establish the severity of hepatitis in the individual (Conry-Cantilena 1997).

2.2.5 Biomarkers for fibrosis

Assays for biochemical markers of fibrosis may offer an alternative to liver biopsy for assessment of liver fibrosis. These assays include procollagen type III peptide, type IV 7s collagen, and hyaluronan, peptides that have been observed to increase in the sera of patients with chronic liver disease and to correlate with the degree of liver fibrosis. In a study of patients with chronic hepatitis and cirrhosis, the ratio of metalloproteinases (which degrade collagens) to tissue inhibitors of metalloproteinase was significantly higher in the sera of patients who did not respond to treatment or responded transiently than in the sera of those with a sustained response. This study suggests that these proteins may be useful biomarkers for predicting responses to interferon treatment in patients with chronic HCV infection (Kasahara *et al.* 1997).

2.3 Epidemiology of infection

2.3.1 HCV prevalence and incidence

2.3.1.1 Geographical and temporal trends

The estimated worldwide prevalence of HCV seropositivity is 3%, affecting approximately 170 million individuals; however, both the prevalence and incidence vary geographically and temporally. Prevalence measurements are usually based on studies of blood donors; these studies use EIA assays to measure antibodies against HCV (see Section 2.2.1). Studies in blood donors probably underestimate exposure, because blood donors are a highly selected population who are usually at a lower risk for blood-borne disease because of rigorous screening policies. HCV prevalence is very low (0.01% to 0.1%) in the United Kingdom and Scandinavia; low (0.2% to 0.5%) in Western Europe, North America, and most of Central and South America, Australia, and limited regions of Africa; intermediate (1% to 5%) in Brazil, Eastern Europe, the Mediterranean, the Middle East, the Indian subcontinent, and parts of Africa and Asia; and highest (17% to 26%) in Egypt. Prevalence rates are unknown for much of Africa and parts of South America (Wasley and Alter 2000). The high prevalence of HCV in Egypt may be a result of treatment programs for schistosomiasis conducted from the 1960s through the 1980s. The treatment consisted of parenteral therapy, usually involving 12 to 16 tartar emetic injections. Evidence suggests that inadequately sterilized needles may have been used, contributing to the transmission of HCV (Rao *et al*. 2002, Frank *et al.* 2000).

Studies evaluating age-specific HCV prevalence have identified three geographical age-related profiles, suggesting three different transmission patterns (see Figure 2-1). Age-specific patterns apply only to a particular calendar time. In the first pattern, which is observed in the United States (see Section 2.3.1.2), HCV prevalence is low in people less than 20 years of age, increases through middle age, and then declines sharply in people over 50, suggesting that most HCV infections occurred in the recent past among young adults. In the second pattern, which is observed in Japan and Italy, prevalence is low in children and young adults but rises sharply among older persons, suggesting that most infections occurred 30 to 50 years ago. HCV prevalence increases steadily with age and is high in all age groups in the third pattern, which is observed in Egypt, indicating a high rate of both past and recent infection (Wasley and Alter 2000).

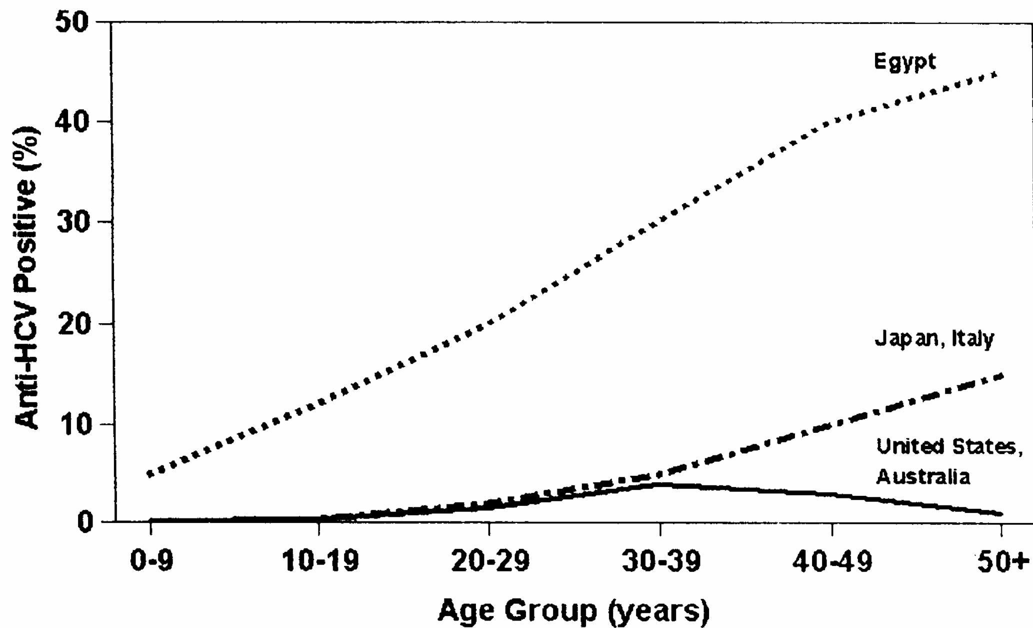

Figure 2-1. Geographical differences in the age-specific prevalence of anti-HCV antibodies

Source: Wasley and Alter 2000.

2.3.1.2 Incidence and prevalence in the United States

The incidence of acute hepatitis C in the United States between 1960 and 1999 is plotted in Figure 2-2. The incidence of newly acquired HCV infection has declined substantially in the last decade, from 180,000 to 30,000, probably as a result of testing of blood donors and decreased numbers of cases among illegal intravenous drug users (see Figure 2-2). Several studies in the United States conducted from 1990 to 1996 among healthy volunteer blood donors have reported the prevalence of HCV antibodies to range from 0.17% to 1.4%; four of the five studies reported prevalence estimates below 0.5% (Memon and Memon 2002). Glynn *et al.* (2000) reported that prevalence in first-time blood donors decreased significantly, from 0.63% to 0.40% ($P < 0.001$) in a study of 1.9 million blood donors at five blood centers in different regions of the United States. As discussed above, these studies in blood donors probably underestimate the prevalence of HCV.

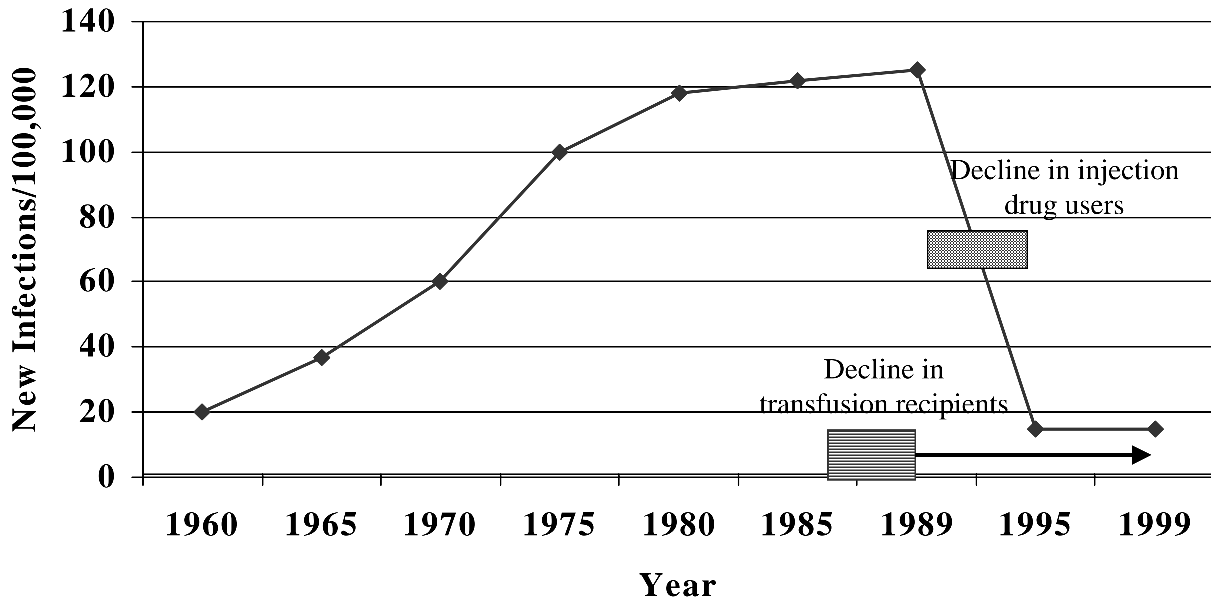

Figure 2-2. Estimated incidence of acute hepatitis C infection: United States, 1960 to 1999

Sources: Armstrong *et al.* 2000, Alter 1997

The largest population-based assessment of HCV prevalence completed to date used samples from 21,241 people collected from 1988 through 1994 as part of the third National Health and Nutrition Examination Survey (NHANES III) (Alter *et al.* 1999). The prevalence of anti-HCV antibodies in the total U.S. population was 1.8% (95% CI = 1.5% to 2.3%), which extrapolates to approximately 3.9 million people. Of people infected with HCV, 65% were between the ages of 30 and 49, and the prevalence was less than 1% in individuals younger than 20 or older than 59 (as discussed above; see Figure 2-1). The prevalence of HCV RNA positivity among those testing positive for anti-HCV antibodies was 73.9%, corresponding to an estimated 2.7 million people chronically infected with HCV. Although some differences in prevalence of HCV infection were noted among ethnic groups (i.e., non-Hispanic whites, non-Hispanic blacks, and Mexican Americans), neither sex nor racial-ethnic group was independently associated with HCV infection. This study also calculated prevalence and odds ratios (ORs) for potential risk factors; however, the study did not ascertain intravenous drug use or transfusion history (these findings are discussed in Section 2.3.3.1 and 2.3.3.2). The authors suggested that their estimates of prevalence could be considered conservative because some groups with high rates of HCV infection (i.e., incarcerated and homeless

persons) were excluded from NHANES III. They also tested only a single sample from each subject, which could miss some individuals who intermittently test positive for HCV RNA. Several studies have reported that HCV prevalence is higher in homeless populations (5% to 32%) (Noell *et al.* 2001, Rosenblum *et al.* 2001) and in veterans (7% to 42%, with the higher rates observed in homeless veterans) (Cheung 2000, Cheung *et al.* 2002); most of this higher prevalence is probably explained by illegal intravenous drug use (see Section 2.3.3.1)

2.3.1.3 Prevalence of HCV genotypes

As described in Section 1.1.2, HCV can be grouped into six major clades (groups of closely related genotypes). The distribution of these genotypes varies geographically. Genotypes 1a and 1b are predominant in the United States and Western Europe, genotypes 2, 4, and 5 in Africa, genotypes 1, 3 and 6 in Southeast Asia, and genotype 4 in Egypt (Poovorawan *et al.* 2002).

The NHANES III study from the United States also measured genotype distribution in 250 of 283 HCV-RNA-positive samples. Genotype 1a occurred in 56.7%, 1b in 17.0%, 2a in 3.5%, 2b in 11.4%, 3a in 7.4%, 4 in 0.9%, and 6 in 3.2% (Alter *et al.* 1999). Genotype frequency was found to vary by geographic region within the United States in a study of 6,807 patients with chronic HCV being treated at 636 clinical centers. Genotype 1 was identified in a significantly ($P < 0.001$, χ^2 analysis) higher percentage of patients from the Northeast, Southeast, and Midwest than from the West or South. Infection with genotype 1 also was more likely to be detected in African-American patients than in Caucasians, Hispanics, or Asian Americans and Pacific Islanders (Blatt *et al.* 2000).

2.3.2 Risk factors

Figure 2-3 shows the proportions of acute adult hepatitis C cases by selected risk factors, based on surveillance studies conducted by the Centers for Disease Control and Prevention (CDC) from 1983 to 1996. The major risk factor for acquiring HCV was illegal intravenous drug use, which accounted for approximately 60% of cases in adults. Only 3% of cases resulted from blood transfusion; however, before the testing of blood, transfusions accounted for 20% of HCV infections. Other risk factors included healthcare employment in patient care or laboratory work, exposure to a sexual partner or household member with a history of hepatitis, and exposure to multiple sexual partners; however, no risk factors were identified for at least 10% of patients with acute HCV. Human studies on these major risk factors are described in detail under the relevant routes of transmission (Section 2.3.3) (Major *et al.* 2001).

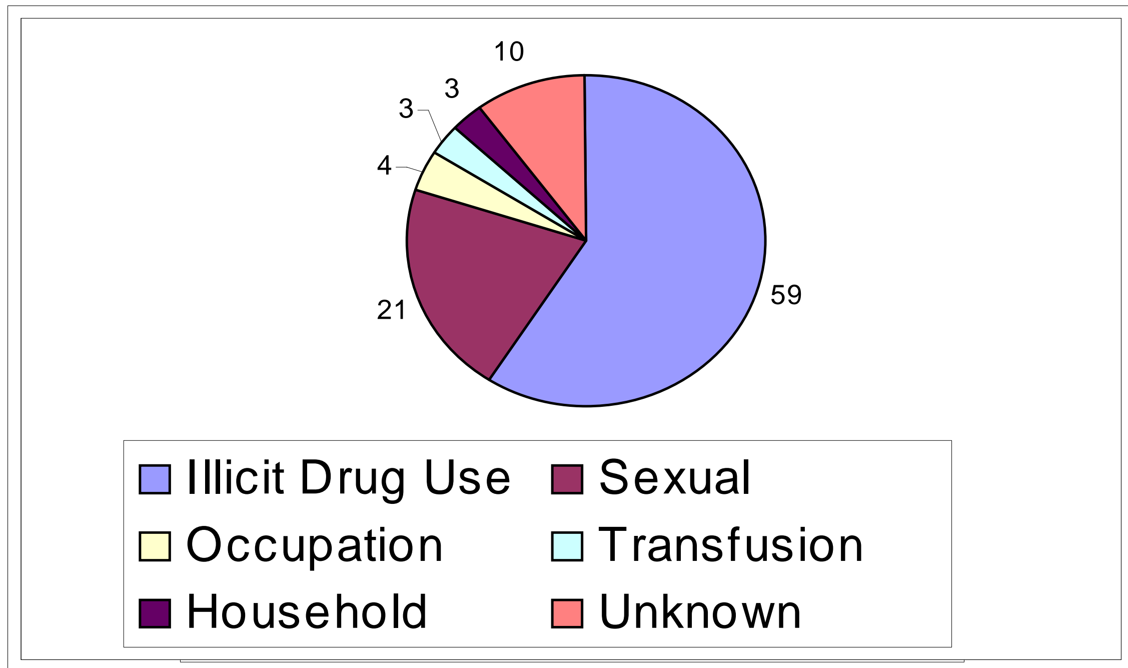

Figure 2-3. Proportions of adult acute hepatitis C cases related to selected risk factors in the United States (1983 to 1996)

Source: Major *et al.* 2001

As discussed in Section 2.3.1.2, the NHANES study did not ascertain illegal intravenous drug use or transfusion history but did calculate the prevalence and multivariate ORs for other selected risk factors (Alter *et al.* 1999). Increased prevalence of HCV infection was associated with a history of cocaine or marijuana use and with an early age at first sexual intercourse, a greater number of sexual partners, and infection with herpes simplex virus type 2. The strongest independent associations (determined by multivariate analysis) with HCV infection among persons 17 to 59 years of age were illegal drug use (ever having used cocaine or ≥ 100 lifetime uses of marijuana) and high-risk sexual behavior (defined as an early age of first intercourse or ≥ 50 lifetime sexual partners). Other factors independently associated with HCV infection were marital status, income (either above or below the poverty level), and number of years of education. The authors proposed that marijuana use could serve as a surrogate for potential methods of transmission such as intravenous drug use and high-risk sexual practices. Similarly, the intranasal use of cocaine could involve sharing of blood-contaminated straws; however, cocaine use could also be related to intravenous drug use. More data are needed to determine whether intranasal cocaine use itself is a risk factor for HCV infection (Wasley and Alter 2000).

2.3.3 Transmission

For transmission of HCV to occur, infectious virus must contact susceptible tissue (Thomas 2001). HCV RNA is found most frequently in blood, and there are conflicting data concerning the detection of virus in other body fluids, such as semen, saliva, and vaginal secretions (Memon and Memon 2002). HCV is transmitted primarily by exposure

to infected blood (parenteral transmission); thus, the major routes of transmission are intravenous drug use and transmission of blood or blood components. Transmission also can occur in healthcare settings. Parenteral transmission of HCV appears to be 10 times as efficient as that of human immunodeficiency virus (HIV), based on studies comparing incidence rates in individuals occupationally exposed through needlestick injury (Kiyosawa *et al.* 1991, Mitsui *et al.* 1992). As shown in Figure 2-3, blood-related routes of transmission only explain 60% to 70% of the cases of acute hepatitis; other postulated routes of transmission are sexual, familial, and perinatal (mother to infant).

2.3.3.1 Illegal drug use and transmission of HCV

The prevalence of HCV infection is highest among intravenous drug users; 27 studies conducted in various geographical locations between 1989 and 1991 reported the prevalence of anti-HCV antibodies in intravenous drug users to range from 31% in Hanoi, Vietnam, to 98% in Denmark. The numbers of individuals tested ranged from 58 to 2,462. Prevalence rates in the United States range from 67% to 85% (Memon and Memon 2002). Cohort studies of intravenous drug users in the United States have reported incidence rates from 6.4 to 16 per 100 person-years (Thorpe *et al.* 2002, Villano *et al.* 1997, Garfein *et al.* 1998). Risk factors identified for the presence of anti-HCV antibodies in intravenous drug users include duration of drug use, male gender, age, working in a sex trade, risky sexual behavior, multiple sexual partners, needle sharing, and a history of imprisonment. When illegal drug use was by the oral route, the risk was of HCV infection was no more than about one tenth that associated with illegal intravenous drug use in the same study (Memon and Memon 2002). Intravenous drug use appears to be the dominant mode of transmission in countries where most infections have occurred in young adults in the recent past (the first age-specific pattern described in Section 2.3.1.1). In contrast, in countries where increased incidence of HCV infection occurred farther in the past (the second and third age-specific patterns), healthcare-related procedures appear to be the dominant mode of transmission (see Section 2.3.3.3) (Wasley and Alter 2000).

2.3.3.2 Transfusion of blood and blood products and organ transplants

In most developed countries, infectious disease screening and testing practices have eliminated most transfusion- and transplant-related transmission of HCV (Wasley and Alter 2000). Since the 1980s, the risk of transmission has decreased with improvements in screening tests (see Sections 2.2.3 and 2.3.1.2). The overall HCV infection rate in repeat blood donors between 1991 and 1996 was 3.25 per 100,000 person-years, which is somewhat higher than that of HIV (2.92), HTLV (1.59), or hepatitis B surface antigen (HBsAg) (2.66). From 1991 to 1996, the HCV infection rate in U.S. blood donors decreased; this decrease was not related to detection methods, because all samples were tested by second-generation EIA (Glynn *et al.* 2000).

The incidence of post-transfusion HCV infection is related to the number and amount of blood products received; for example, hemophiliacs had a high incidence of HCV infection (46% to 90%). Virus-inactivating procedures for blood products, such as heat treatment, pasteurization, and solvent-detergent treatment, have nearly eliminated the risk of HCV transmission by this route (Memon and Memon 2002).

2.3.3.3 Transmission related to healthcare practices (nosocomial and occupational)

The prevalence of HCV in hospital-related settings has been reported to range from 2% to 18%. Prevalence among patients in chronic hemodialysis clinics is approximately 20%, though rates vary greatly geographically; low rates (1% to 5%) have been observed in the United Kingdom and South Africa, intermediate rates (10% to 50%) in North America, Scandinavia, and Western and Southern Europe, and high rates in Eastern Europe (20% to 91%) (Wasley and Alter 2000). Routes of transmission in these patients include blood transfusions, sharing of dialysis machines between HCV-positive and -negative patients, and nosocomial transmission by dialysis staff (Memon and Memon 2002). The incidence of HCV infection in hemodialysis patients without any identified risk factors is estimated to be approximately 2% to 2.6% per year (Wasley and Alter 2000). In the United States, CDC estimated the incidence to be 0.6 in 1993.

Nosocomial transmission of HCV in healthcare-related settings can occur from patient to patient, from infected healthcare worker to patient, and from healthcare-related practices; however, the risk from the first two routes appears to be very low. Most cases of patient-to-patient transmission appear to involve unsafe injection practices resulting in contaminated equipment. Similarly, there have been very few reports of HCV transmission from healthcare workers to patients. The length of time on hemodialysis has consistently been found to be an independent predictor of HCV infection (Fabrizi *et al.* 1998). Studies of dialysis-associated outbreaks indicated that transmission probably occurred as a result of incorrect implementation of infection-control procedures, resulting in opportunities for cross-contamination between patients; these practices include sharing of equipment and supplies, failure to disinfect equipment between patient uses, sharing of medication vials, and contamination of priming buckets, which were not disinfected or cleaned between patients. Healthcare-related procedures may have been an important mode of transmission in countries where most of the HCV prevalence occurred 30 to 50 years ago (the second age-specific pattern discussed above) and where the risk has been high for many decades (the third age-specific pattern) (Wasley and Alter 2000).

HCV also can be transmitted from patient to healthcare worker (occupational transmission); however, this risk appears to be small, and the prevalence of HCV infection among healthcare workers in the United States appears to be similar to that of the general population (0.6% to 1.9%); however, in a study in an area with high prevalence of acquired immunodeficiency syndrome (AIDS), the prevalence of HCV in healthcare workers was 5.3% (Memon and Memon 2002). High-risk groups include surgeons, obstetricians, hemodialysis nurses and technicians, oral surgeons, emergency department workers, and intensive care workers. Risk factors include type of needle, frequency of occupational blood contacts, type of patient (chronic vs. acute), and prevalence of HCV among the patients (Memon and Memon 2002). The major route appears to be needlesticks; however, transmission via blood splashes to the eye has been reported (Wasley and Alter 2000). The average seroconversion from needlesticks is 1.8%, although higher rates have been reported in studies in Japan (3.3% and 5.6%) (Memon and Memon 2002, Wasley and Alter 2000).

2.3.3.4 Sexual transmission of HCV

The importance of sexual activity as a transmission route for HCV is controversial. Evidence for sexual transmission of HCV was reviewed by Wasley and Alter (2000), who concluded that the available evidence supported sexual transmission, but that this route was inefficient. CDC's finding that 15% to 20% of HCV cases in the United States were attributable to sexual exposure in the absence of intravenous drug use (see Figure 2-3) may have resulted from studying a large population with multiple partners and a large population of HCV-infected individuals (Wasley and Alter 2000).

Supporting the role of sexual transmission are case-control and cross-sectional studies reporting an association between sexual activity and HCV and the NHANES general population study, which reported that high sexual activity was an independent risk factor for HCV (see Section 2.3.2) (Wasley and Alter 2000, Alter *et al.* 1999). A relatively high prevalence of HCV infection has been reported for heterosexual individuals attending clinics for sexually transmitted disease, male homosexuals, prostitutes, and partners of illegal intravenous drug users. It has been suggested that the following factors are positively correlated with HCV infection: overall number of sexual partners, not using a condom, receptive anal intercourse, sexual activity involving a trauma, history of other sexually transmitted diseases, and infection with HIV (Memon and Memon 2002). There is some evidence that male-to-female transmission may be more efficient than female-to-male transmission (Wasley and Alter 2000).

Other studies of homosexual men and long-term steady sex partners of people chronically infected with HCV suggest that sexual activity may not be an important route of transmission. In most studies at sexually transmitted disease clinics, prevalence rates among men having sex with other men are similar to those of heterosexuals, although sexual transmission of blood-borne viruses is recognized to be more efficient in the former. Bresters *et al.* (1993) did not detect any evidence of sexual transmission in a study of 50 heterosexual partners of HCV-infected individuals with a median duration of the sexual relationship of 13 years. Moreover, the prevalence of HCV in spouses of HCV-infected persons who report no other risk factors for infection is low (1.3%) in Western Europe and North America. In Asia, the prevalence in spouses of HCV-infected persons (with no other risk factors) is much higher (27%), but this finding could be a result of percutaneous exposures shared by both spouses, especially in countries where use of contaminated equipment in medical procedures was common (Wasley and Alter 2000).

2.3.3.5 Familial transmission of HCV (household contacts)

The prevalence of anti-HCV seropositivity has been reported to be 5- to 10-fold higher in individuals living with an HCV-positive patient than in the general population (Memon and Memon 2002). Nonsexual transmission of HCV has been proposed for these situations; however, spouses are more likely to be affected than are children. It has been speculated that nonsexual familial transmission might occur through sharing of toothbrushes, dental appliances, razors, and nail-grooming equipment.

2.3.3.6 Perinatal transmission

Perinatal transmission (mother to infant) accounts for a small proportion of total HCV infections worldwide; however, it is the major risk factor for HCV transmission in children (Schwimmer and Balistreri 2000). Perinatal transmission occurs almost exclusively in women who are HCV-RNA positive at the time of delivery. There appears to be little risk to infants of women who are anti-HCV positive but HCV-RNA negative. Data from 25 studies following infants for at least 12 months reported an average transmission rate of 6% (range 0% to 42%) for infants born to HCV-infected women not infected with HIV and 17% (range 8.5% to 44%) for infants born to women coinfected with HCV and HIV. Virus titers appear to be associated with HCV transmission by women infected with both viruses, but the evidence is conflicting in studies of women infected only with HCV. Delivery method and breastfeeding do not appear to affect the HCV transmission rate. The average rate of HCV infection among infants was similar for infants born by vaginal delivery (10%) and cesarean section (8.4%), and the average infection rate was similar among breast-fed infants (5%) and bottle-fed infants (8%) (Wasley and Alter 2000).

2.3.4 Natural history of hepatitis C virus infections

HCV is a major public health problem globally. The European Association for the Study of the Liver reported that new symptomatic infections of HCV are estimated at 1 to 3 cases per 100,000 persons per year. They inferred a much higher actual incidence of new infections, because the majority of cases are asymptomatic (EASL 1999). Worldwide, HCV is the leading cause of chronic liver disease and is a major cause of liver transplants, cirrhosis, and hepatocellular carcinoma (Boyer and Marcellin 2000). HCV-related liver disease may account for 8,000 to 10,000 deaths per year in the United States. As of 1994 (NHANES study), most HCV-infected individuals were between 30 and 49 years of age; thus, the number of deaths could substantially increase during the next 20 to 30 years, as this group reaches the age at which complications from liver disease usually occur (MMWR 1998).

2.3.4.1 Acute hepatitis

Persons with acute hepatitis are asymptomatic (60% to 70%) or have mild clinical disease symptoms; 10% to 20% have nonspecific symptoms, such as nausea, vomiting, anorexia, or abdominal pain, and 20% to 30% may become jaundiced. The average time from exposure to symptoms is six to seven weeks. After initial exposure, HCV RNA can be detected in the blood within one to two weeks after exposure. The average time for seroconversion is eight to nine weeks; thus, some individuals have symptoms before seroconversion. Anti-HCV antibodies are detected in 50% to 70% of individuals at the onset of symptoms and in over 97% by six months after exposure (MMWR 1998).

ALT levels (liver enzymes) usually are elevated and often display a fluctuating pattern. A decrease of ALT to normal levels can mean recovery, but often this decrease is followed by progression to chronic disease. Hepatic histopathologic changes in acute HCV infection and other acute viral hepatitis include fatty change in hepatocytes, pleomorphism of hepatocytes, lobular necrotic foci and inflammation, acidophilic (apoptotic) bodies, and portal tract inflammation with interface hepatitis (erosion of the

limiting plates). The fatty change in hepatocytes is more common with HCV infection than with other types of viral hepatitis (Bonkovsky and Mehta 2001).

2.3.4.2 Resolution and clearance

Complete resolution of HCV infection is defined as normalization of serum ALT levels and absence of viremia (i.e., HCV RNA) in the blood (Schouten 2000). Viral clearance is assumed to occur when HCV cannot be detected in someone with anti-HCV or in someone in whom acute infection was observed; clearance occurs in approximately 10% to 25% of individuals after acute infection (Kondili *et al.* 2002, Thomas *et al.* 2000). Thomas *et al*. (2000) studied the natural history of HCV infection in a community-based, prospective cohort study in the United States of 1,667 individuals with a history of intravenous drug use and a positive test for anti-HCV antibodies during follow-up (median = 8.8 years). Viral clearance was observed in 11% of the individuals and was more likely to occur in non-blacks, females, HIV-negative individuals, and HBsAg-positive individuals. Other factors shown to be associated with viral clearance include younger age, female gender, certain histocompatibility complex genes, jaundice (suggesting more severe acute hepatitis), and a lower viral titer (NIH 2002, Bonkovsky and Mehta 2001). Clearance rates have been reported to range from 30% to 50% in children (Schwimmer and Balistreri 2000). Viral clearance may also be related to a decrease in viral diversity over time. HCVs are quasispecies in nature; thus, multiple viral strains have been isolated from the same individual. Studies have shown that antibodies against HCV are strain-specific and thus incapable of preventing the evolution of viral variants that maintain an infection (Busch 2001). A study of 12 acutely infected post-transfusion patients suggested that viral persistence was related to genetic evolution of the virus in the first four months following infection (Farci as cited by Busch 2001).

Seroreversion rates have been reported to range from 7% to 19% in cohort studies of various populations, such as post-transfusion patients and the general population; the highest seroreversion rate was observed in the general population in an area in Italy where the risk of HCV infection was low (Kondili *et al.* 2002, Seeff *et al.* 2001).

2.3.4.3 Chronic hepatitis

Chronic HCV develops in 75% to 80% of individuals with acute hepatitis C infection (Bonkovsky and Mehta 2001). Chronic hepatitis is usually defined as persistently abnormal ALT levels for at least six months; however, some individuals have normal ALT levels but have other evidence of persistent infection, such as detectable HCV RNA in the serum (for at least six months) and other histopathologic abnormalities observed in liver biopsies (Bonkovsky and Mehta 2001). Individuals with chronic hepatitis are the source for all new infections and are at an increased risk for chronic liver disease, cirrhosis, and hepatocellular carcinomas. Many individuals do not have symptoms, others have fatigue as the only symptom, and others have overt symptomatic liver disease with anorexia, nausea, right upper quadrant pain, dark urine, and pruritis (Major *et al.* 2001).

The National Heart, Lung, and Blood Institute (NHLBI) reported the results from a 25-year retrospective series of three studies consisting of 90 individuals with transfusion-associated hepatitis C. Of these individuals, 7% had cleared their infection (no markers of

HCV were present), 17% had antibody in the absence of viremia, and 77% were chronically viremic. Of the individuals with chronic viremia, 49% had biochemical evidence of chronic hepatitis (38% of the original cohort) (Seeff *et al.* 2001). Alter *et al.* (1992) conducted a follow-up study (9 to 48 months) of community-acquired HCV and found that 62% of the HCV-infected individuals developed chronic hepatitis infection, which was not related to the risk factors for infections; that is, the percentage of individuals developing chronic hepatitis was similar regardless of the method of transmission (parenteral exposure, such as by blood transfusion or intravenous drug use, or non-parenteral exposure, such as by sexual or familial contact).

Chronic hepatitis can progress into progressive liver fibrosis, leading to cirrhosis, end-stage liver disease, and hepatocellular carcinoma. In the United States, HCV is the primary reason for liver transplantation and causes one-third of the cases of hepatocellular carcinoma (NIH 2002). Progression occurs in some but not all individuals and occurs very slowly and usually without symptoms or physical signs in the first two or more decades after infection. In the NHLBI study of transfusion-associated hepatitis, clinically evident liver disease was observed in 23% of the individuals with chronic hepatitis, and cirrhosis in 35% (17% of the original HCV-infected individuals) (Seeff *et al.* 2001). Liver disease may be relatively less common in healthy populations. Seeff *et al*. (2000) conducted a 45-year follow-up study in 8,568 healthy adult military recruits. Liver disease occurred in only 11.8% of the HCV-infected individuals; however, because HCV prevalence was very low in this population (0.2%), there were only 17 HCV-infected individuals (2 with liver disease).

In those infected with HCV, cirrhosis is generally considered to develop in 10% to 20%, and hepatocellular carcinoma in 1% to 5%. Based on data from several studies of patients with transfusion-related hepatitis C infection referred for evaluation of liver disease, the mean interval from infection to diagnosis was 13.7 years for chronic hepatitis, 18.4 years for chronic active hepatitis, 20.6 years for cirrhosis, and 28.3 years for hepatocellular carcinoma (Major *et al.* 2001). Progression to serious liver disease probably is not related to virologic factors (such as viral load and genotype) but probably is related to host factors such as age, male gender, and immunosuppressed state (such as concurrent HBV or HIV infection). Other factors include heavy alcohol use, iron overload, nonalcoholic fatty liver disease, schistosomal coinfection, potentially hepatotoxic medication, and environmental contaminants (Bonkovsky and Mehta 2001, NIH 2002).

Although most children with perinatally acquired HCV develop chronic disease, most are asymptomatic, suggesting milder disease. However, a few children do develop cirrhosis (Schwimmer and Balistreri 2000).

2.3.4.4 Extrahepatic manifestations of hepatitis C infection

Chronic HCV also may cause extrahepatic manifestations such as porphyria cutanea tarda and syndromes that may be of immunologic origin, including rheumatoid symptoms, keratoconjunctivitis sicca, lichen planus, glomerulonephritis, and essential mixed cryoglobulinemia. Extrahepatic effects of HCV include many types of immune-mediated manifestations, including autoimmune disorders and abnormal production or deposition of B-cell immunoglobulins (Bonkovsky and Mehta 2001).

2.4 Therapy and vaccines

2.4.1 Treatment

In a review of therapies for HCV infection, McHutchison *et al.* (2002) reported that the combination of interferon and ribavirin is the most effective initial therapy for patients who are considered suitable for treatment. The diagnostic criteria for such patients include elevated liver enzymes, detectable HCV RNA, and liver biopsy demonstrating significant inflammation or fibrosis. A successful response to therapy is considered to be a sustained viral response, defined as undetectable serum HCV RNA at least six months after the end of therapy. This type of response is usually associated with a return of serum aminotransferases to normal levels, and improvement in liver inflammation. Recent evidence suggests that successful antiviral therapy also may be associated with a decreased degree of hepatic fibrosis (Poynard *et al.* 2002).

A relatively new formulation of interferon-α-2a has been developed, in which the interferon molecule has been modified by a covalent bond to polyethylene glycol (peginterferon-α-2a); this modification prevents enzymatic degradation, thus reducing systemic clearance and enabling administration once, rather than three times, per week (Perry and Jarvis 2002). From their review of the literature, Perry and Jarvis (2002) reported that peginterferon-α-2a was significantly more effective than interferon-α in patients with and without cirrhosis in achieving a sustained virological response and in producing beneficial histological effects on the liver. The recent NIH Consensus Statement (NIH 2002) favors the combination of peginterferon and ribavirin as the optimal therapy against hepatitis C. It has been suggested that interferon-based therapies may reduce the risk of hepatocellular carcinoma, particularly in patients with a sustained virologic response.

Research to develop new strategies for treatment of hepatitis C includes investigation of potential ways to directly inhibit HCV proteins or to interfere with viral replication in other ways (Cornberg *et al.* 2001). Proteins encoded by HCV RNA that are essential to viral replication, and thus are potential targets for drug design, include the NS2/3 metalloproteinase, the NS3 [+NS4A] serine protease, the NS3 helicase/NTPase, the NS5B polymerase, and the 5' UTR (internal ribosome entry site) (Locarnini and Bartholomeusz 2002). The potential also exists for treatment based on inhibition of viral replication or gene expression through HCV-specific ribozymes that cleave HCV RNA or antisense oligonucleotides that bind to specific HCV RNA sequences to block the replication, reverse transcription, or mRNA translation of HCV RNA (Idéo and Bellobuono 2002).

2.4.2 Vaccines

No effective vaccine against HCV exists (Prince 2001). Prince (2001) suggested that the failure of vaccine tests in animals to demonstrate any protective or therapeutic effects despite impressive immune responses could be due to suboptimal immunogenicity of HCV or to the ability of the virus to escape from the immune response through its high rate of genetic variation and multiple quasispecies.

2.5 Universal precautions

The Occupational Safety and Health Administration (OSHA) on January 1, 1992 (OSHA 1992) established a "bloodborne pathogens final standard" covering potential exposure to infectious material, including semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate among body fluids. This standard also included unfixed human tissues or organs and any cell, tissue, or organ cultures containing HIV or HBV, including those from experimental animals infected with either of these viruses (see Section 2.6 and Table 2-4). Subsequent recommendations from the U.S. Public Health Service have included HCV in recommendations on occupational exposures to bloodborne pathogens (CDC 2001).

The central feature of the OSHA standard is the concept of universal precautions, which are emphasized in medical and laboratory settings throughout the United States. The principle of universal precautions calls for all body fluids and materials to be treated as infectious and establishes specific practices to be followed. These practices include provisions for handwashing and procedures to minimize needlesticks and splashing and spraying of blood, procedures to ensure appropriate packaging of specimens and regulated wastes, and instructions to ensure proper labeling or decontamination of contaminated equipment. In addition, employees must have access to and use personal protective equipment, such as gloves, gowns, masks, mouthpieces, and resuscitation bags, to reduce the potential for contact with body fluids. The standard specifies methods for safely disposing of contaminated sharps and sets forth standards for containers for these items and other regulated waste. Microbiological laboratories must follow standard microbiological practices and additional practices intended to minimize exposure of employees working with concentrated viruses and to reduce the risk of accidental exposure of other employees at the facility. Employers also are required to provide the following: (1) a written schedule for decontamination following contact with potentially infectious materials, (2) hazard communication through orange or orange-red biohazard symbols on warning labels of containers with potentially infectious materials, (3) information and training of employees, (4) HBV virus vaccination within 10 working days of assignment, (5) post-exposure evaluation and follow-up, including a confidential medical evaluation, and (6) maintenance of medical records for each employee with occupational exposure.

2.6 Regulations

The FDA has established (1) regulations for human blood and blood products that require assessing whether the source of whole blood has a history of viral hepatitis and (2) regulations requiring that human tissue donors be assessed for their risk of hepatitis and that specimens from the donors be tested for hepatitis C. OSHA has established rules for the recording of occupational injuries that could lead to hepatitis C infection. In addition, OSHA regulations for vinyl chloride, a known liver toxicant, require that a medical surveillance program be undertaken for each employee exposed to vinyl chloride above the action level. This program requires that the employee's medical history be

taken, including any past history of hepatitis. See Tables 2-3 and 2-4 for a summary of these regulations.

Table 2-3. FDA regulations

Regulatory citation	Regulatory action
21 CFR 640 - PART 640 - ADDITIONAL STANDARDS FOR HUMAN BLOOD AND BLOOD PRODUCTS. Promulgated: 38 FR 32089, 11/20/73. U.S. Codes: 21 U.S.C. 321, 351, 352, 353, 355, 360, 371; 42 U.S.C. 216, 262, 263, 263a, 264.	No individual shall be used as a source of whole blood if he has a history of of viral hepatitis, a history of close contact within 12 months of donation with an individual having viral hepatitis, or a history of having received within 12 months of donation, human blood or any derivative which the FDA has advised the blood establishment is a possible source of viral hepatitis.
21 CFR 1270 - PART 1270 - HUMAN TISSUE INTENDED FOR TRANSPLANTATION. Promulgated: 62 FR 40444, 7/29/97. U.S. Codes: 42 U.S.C. 216, 243, 264, 271.	The potential donor must be questioned to elicit whether he or she is at increased risk for hepatitis and must be physically assessed for any signs of hepatitis infection. The potential donor's medical records must be checked for hepatitis. Donor specimens must be tested for hepatitis C.

The regulations in this table have been updated through the 2001 Code of Federal Regulations, December 31, 2001.

Table 2-4. OSHA regulations

Regulatory citation	Regulatory action
29 CFR 1904 and 1952 - PARTS 1904 AND 1952 - OCCUPATIONAL INJURY AND ILLNESS RECORDING AND REPORTING REQUIREMENTS. Promulgated: 66 FR 5916, 1/19/01. U.S. Codes: 29 U.S.C. 657, 673.	Rules are established for the recording of occupational injuries involving punctures, cuts, and lacerations caused by needles or other sharp objects contaminated with potentially infectious materials that could lead to hepatitis C.
29 CFR 1910 - PART 1910, SUBPART Z - TOXIC AND HAZARDOUS SUBSTANCES. Promulgated: 39 FR 35896, 10/4/74, as amended numerous times. U.S. Codes: 29 U.S.C. 653, 655, 657.	A program of medical surveillance shall be instituted for each employee exposed to vinyl chloride in excess of the action level. A medical history shall be taken, including the employee's past history of hepatitis. Universal precautions should be observed to prevent contact with blood or other potentially infectious material.

The regulations in this table have been updated through the 2001 Code of Federal Regulations, December 31, 2001.

2.7 Summary

HCV infection is usually assessed by detection of antibodies against HCV proteins or by detection of HCV RNA. Anti-HCV antibodies are detected by serological assays, which have undergone a series of improvements resulting in greater sensitivity and specificity. HCV RNA is usually detected by PCR-based tests.

The worldwide prevalence of HCV seropositivity is approximately 3% (170 million individuals); however, the prevalence varies geographically. Reported prevalence rates are low (less than 0.5%) in Western Europe, North America, Australia, most of Central and South America, and parts of Africa; intermediate (1% to 5%) in Eastern Europe, the Middle East, the Mediterranean, and parts of Africa and Asia; and highest (17% to 26%) in Egypt. In the United States, approximately 3 to 4 million individuals are infected with HCV. The annual incidence of HCV infection has decreased in the last decade from 180,000 to 30,000.

The major route of HCV transmission is through contaminated blood. The major risk factor for infection is illegal intravenous drug use, which from 1983 to 1996 accounted for almost 60% of acute HCV infections in adults. Since the initiation of screening of blood and blood products in the 1990s, blood transfusion has accounted for only a small percentage of adult HCV cases (~3%). Other routes of transmission include sexual, perinatal, familial (at low rates), and through healthcare practices, including transmission by contaminated equipment or supplies, from patient to patient (at low rates), and through occupational exposure (at low rates).

HCV is the leading cause of known liver disease in the United States. HCV infection can result in chronic hepatitis, cirrhosis, or liver cancer. Currently, HCV is treated by interferon-related therapies, and no vaccine is available.

3 Human Cancer Studies

3.1 Introduction

HCV-infected individuals are at increased risk for two forms of cancer, hepatocellular carcinoma and B-cell lymphoma. Some salient features of the epidemiology of the two HCV-related cancers are briefly described below.

3.1.1 Hepatocellular carcinoma

Hepatocellular carcinoma is the primary histological subgroup of primary liver cancer in humans. In the United States, where primary liver cancer is relatively rare, hepatocellular carcinoma constitutes 70% to 75% of primary liver cancer cases. In East and Southeast Asia and sub-Saharan Africa where primary liver cancer is one of the most commonly occurring cancers, hepatocellular carcinoma constitutes well over 90% of primary liver cancers (Parkin *et al.* 1997).

Hepatocellular carcinoma is a disease with a strong male predominance, which is observed across all populations. Rates are at least two to three times as high in men as in women, and this sex ratio is especially pronounced in high-risk regions (Yeh *et al.* 1989, Parkin *et al.* 1997).

In the United States, the incidence of hepatocellular carcinoma has been increasing steadily over the past two decades; the overall age-adjusted incidence during the period 1991 to 1995 was approximately 70% higher than the rate during the period 1976 to 1980. Rates of increase have been comparable between black and white men and more pronounced in men than in black or white women (El-Serag and Mason 1999). These authors speculate that these rates of increase may be explained by the demographics of the study population. The majority of the study population was 12 to 49 years of age during the 1960s and 1970s, when several risk factors for the transmission of HCV and HBV (e.g., intravenous drug use, needle sharing, transfusion of unscreened blood and blood products, unsafe sexual practices) were prevalent. In HCV- or HBV-infected individuals, the latency period for developing hepatocellular carcinoma is 10 to 30 years; therefore, those who were infected during the 1960s or 1970s would be most likely to develop hepatocellular carcinoma during the 1980s and 1990s, the period when the increase in cancer cases began. If HCV and HBV infections are responsible for the increased hepatocellular carcinoma incidences, the gender and racial differences evident in the United States may be explained by the fact that black Americans are at greater risk for HBV and HCV infection than white Americans, and both HBV infection and HCV infection are more common in men than in women (El-Serag and Mason 1999).

In addition to HCV, HBV is responsible for large numbers of hepatocellular carcinoma cases in humans. Strictly speaking, chronic HBV infection should be defined by two positive results of serum HBsAg tests taken six months apart. In epidemiological studies, obtaining two blood samples is highly impractical. Given that adult non-carriers of HBV are highly unlikely to test positive for HBsAg, a single positive test result for HBsAg is considered a valid indicator of chronic carrier status. The assays of choice (i.e., recognized to be both highly sensitive and specific) for measuring serum HBsAg are

radioimmunoassay (RIA) and EIA. The reverse passive hemagglutination (RPHA) test is considered to be less sensitive than RIA or EIA.

Other serologic markers of HBV infection commonly measured in epidemiologic studies are antibodies to the hepatitis B core antigen (anti-HBc, a marker of past or current infection) and antibodies to HBsAg (anti-HBs, a marker of immunity). Although there is abundant evidence that serologic markers other than HBsAg are not predictive of hepatocellular carcinoma risk in populations with endemic HBV infection, recent data from populations with relatively low infection rates suggest that anti-HBc positivity in the absence of HBsAg and anti-HBs antibodies also predicts hepatocellular carcinoma in these low- to intermediate-risk regions. It is recognized that the presence of anti-HBc antibodies in the absence of other HBV serologic markers can suggest low-level chronic infection.

Established nonviral risk factors for hepatocellular carcinoma include dietary aflatoxin intake, excessive alcohol consumption, and exogenous use of estrogen (oral contraceptives) or androgen. Cigarette smoking has been shown in numerous analytic epidemiologic studies to be a risk factor for hepatocellular carcinoma, independent of alcohol intake. However, given the strong positive correlation between use of tobacco and alcohol in most populations, especially in the West, where both exposures are relatively prevalent, the relationship between cigarette smoking and hepatocellular carcinoma is considered by many to be inconclusive (Yu *et al.* 2000).

3.1.2 B-cell lymphoma

B-cell lymphoma constitutes most cases of non-Hodgkin's lymphoma, a group of diseases comprising various histologic entities with possibly different etiologies. Most epidemiologic data on non-Hodgkin's lymphoma do not differentiate among the subtypes.

Incidence of non-Hodgkin's lymphoma displays an approximately 10-fold variation worldwide. High-risk regions are the developed countries in North America (United States and Canada) and Western Europe, while the underdeveloped countries in Asia and Africa exhibit the lowest rates (Parkin *et al.* 1997).

Non-Hodgkin's lymphoma is a disease with a male predominance, which is observed across all populations. In the United States, rates in men are roughly 50% higher than rates in women of the same ethnicity in the same geographic locations (Parkin *et al.* 1997).

In the United States, the incidence of non-Hodgkin's lymphoma has shown a marked increase over time, more than doubling over the past three decades. This secular increase in incidence is especially pronounced among the elderly (those aged 65 years and older) (Ries *et al.* 2001).

Risk of non-Hodgkin's lymphoma is increased in patients with a variety of conditions involving substantial immune dysfunction, including patients with congenital immunodeficiency, organ or bone marrow transplant recipients, and patients with

acquired immunodeficiency syndrome. Chronic antigenic stimulation of the immune system in these patients is believed to be the underlying cause of increased risk of non-Hodgkin's lymphoma in this subpopulation.

Increased incidence of non-Hodgkin's lymphoma has been reported for several occupational groups with exposure to agricultural chemicals (farmers, herbicide and pesticide appliers, grain workers). Several analytical epidemiologic studies have reported increased risk in subjects exposed to herbicides or pesticides in general or to specific chemicals that are widely used as herbicides and pesticides.

Immune dysfunction, and possibly herbicide exposures, can explain only a small proportion of the non-Hodgkin's lymphoma occurring in the United States. Therefore, the etiology of most cases of non-Hodgkin's lymphoma and the reason(s) for its rising incidence in the United States remain largely unknown (Scherr and Mueller 1996).

3.2 IARC evaluation

IARC convened a Working Group, which met in Lyon, France, from June 8 to 15, 1993, to evaluate the carcinogenic risk to humans of chronic HCV infection (IARC 1994). The Working Group concluded that "there is sufficient evidence in humans for the carcinogenicity of chronic infection with the hepatitis C virus," and HCV was given a Group 1 classification ("the agent is carcinogenic to humans").

The Working Group reviewed three cohort studies (two cohorts of patients with chronic liver disease and a general population cohort) and 23 case-control studies. In all investigations, a positive test result for serum anti-HCV antibodies was used to indicate HCV infection. Most studies reported a significantly increased risk of hepatocellular carcinoma among anti-HCV-positive subjects; relative risk (RR) estimates ranged from approximately 5 to over 100.

In the 12 studies in which risk of hepatocellular carcinoma associated with anti-HCV positivity was examined in the subgroup of HBsAg-negative subjects, HCV infection was positively associated with cancer. A few studies also evaluated other potential risk factors for hepatocellular carcinoma, including alcohol and tobacco use. In all instances, the association between anti-HCV positivity and hepatocellular carcinoma risk was not materially altered after adjustment for possible confounding factors.

3.3 Studies of hepatocellular carcinoma published after the IARC review

Donato *et al.* (1998) undertook a meta-analysis of 32 epidemiologic studies investigating the relationship between coinfection with HBV and HCV and hepatocellular carcinoma risk. These studies are evaluated in Section 3.3.1, below. The remaining studies are discussed separately, by study design (case-control studies in Section 3.3.2 and cohort studies in Section 3.3.3). Whenever applicable, studies are grouped according to study location (Asia, Africa, Americas, Europe).

3.3.1 Meta-analysis of Donato et al. (1998)

For their meta-analysis of 32 studies, Donato *et al.* (1998) selected studies from a Medline search for titles and abstracts of papers published between 1993 and 1997, using

hepatocellular carcinoma and HCV as keywords. For articles published before 1993, they used the IARC monograph literature search. Irrelevant titles and abstracts were eliminated; full-text articles then were obtained, and those without useful information were eliminated. Reports, reference lists, and meeting proceedings and abstracts also were examined for additional literature.

Case-control studies were included in the analysis if the cases included hepatocellular carcinoma patients and controls were subjects without chronic liver diseases (Donato *et al.* 1998). Studies with hepatocellular carcinoma cases only (i.e., without a comparison group) were eliminated, as were studies that selected participants according to HBsAg or anti-HCV seropositivity. Only studies that used the following serological markers of chronic infection were included in the analysis: HBsAg for HBV infection and anti-HCV antibodies (tested with ELISA) or HCV RNA (detected by RT-PCR) for HCV infection. When data were available from both anti-HCV and HCV RNA tests, only the anti-HCV antibody data were used to classify subjects as positive or negative for HCV infection. Reports classifying subjects according to the presence of anti-HBc or anti-HBs antibodies were eliminated, because anti-HBc antibodies are a marker of persistent or past infection, and anti-HBs antibodies typically indicate past infection. Studies with fewer than 10 hepatocellular carcinoma cases were excluded, because they could not detect any subjects who tested positive for both HBsAg and anti-HCV antibodies or HCV RNA. Only studies that reported, or allowed computation of, estimates of the odds ratio (OR) or RR for each combination of HBsAg and anti-HCV or HCV RNA test results were included.

Where several studies of the same population were available, only one, generally the most complete, was used (Donato *et al.* 1998). Any language and country where the study was conducted were acceptable, although abstracts had to be in English for the first evaluation. Unpublished data were accepted if an abstract was available or a report was in press.

Eleven of the 32 studies were part of the IARC evaluation (IARC 1994) and, therefore, are not individually reviewed again here. One study that was described only in an abstract (Yang *et al.* 1996) with no subsequent full-length publication, also is not individually reviewed in this report. Below is a brief description of the design and results (focusing primarily on the association between HCV infection and hepatocellular carcinoma) of the remaining 20 studies; the study results are summarized in Table 3-1. Findings from these studies on the combined effect of HBV and HCV on hepatocellular carcinoma risk in coinfected subjects are reported in Section 3.3.5.

3.3.1.1 Asia

Fukuda *et al.* (1993) studied 368 individuals (287 men, 81 women), aged 40 to 69 years, who were residents of Fukuoka or Saga prefecture and were newly diagnosed with hepatocellular carcinoma at the Kurume University Hospital between April 1986 and May 1992. Controls were inpatients at two university-affiliated general hospitals in Kurume, judged to be free of chronic hepatitis or hepatic cirrhosis and matched to the index cases by age (five-year age groups), sex, residence (prefecture), and time of hospitalization (within two months of the case interview). The case-control ratios were 1:1 for men and 1:4 for women. Serum HBsAg status (tested by RPHA or EIA) was

abstracted from hospital records. Serum anti-HCV status was tested by first-generation EIA in 99 hepatocellular carcinoma patients (70 men, 29 women) and 114 controls (46 men, 68 women). A structured questionnaire was used to interview study subjects in person to obtain information on medical history, family history of liver disease, use of tobacco and alcohol, and usual dietary habits. Of the hepatocellular carcinoma patients, 82.9% men and 82.8% women were anti-HCV positive. The comparable figures in controls were 8.7% of men and 5.9% of women. The matched OR in men for anti-HCV positivity was 35.0 ($P < 0.05$). No matched OR could be calculated for women because no anti-HCV-positive controls were matched to anti-HCV-negative hepatocellular carcinoma patients. Based on tabular data, the lower 95% CI for the matched OR was 4.8.

Cordier *et al.* (1993) conducted a hospital-based case-control study in Hanoi, Vietnam, between 1989 and 1992, involving 152 male patients with hepatocellular carcinoma and 241 male hospital controls of similar ages; the control subjects were admitted to the same hospitals for reasons other than cancer or liver disease. Second-generation EIA was used to assess serum HBsAg and anti-HCV status. A structured questionnaire was used to interview study subjects in person to obtain information on use of tobacco and alcohol, lifetime occupational history, and exposure to pesticides including Agent Orange. The age-adjusted OR for anti-HCV positivity was 2.0 (95% CI = 0.3 to 17.4). Among HBsAg-negative subjects, the comparable OR was 38.1 (95% CI = 2.8 to 1,443).

Pyong *et al.* (1994) conducted a hospital-based case-control study involving 90 Korean patients (68 men, 22 women) newly diagnosed with hepatocellular carcinoma at the Kyowa Hospital, in Osaka, Japan, between January 1989 and December 1992. Controls were 249 Korean patients admitted to the same hospital during the same period; they were between the ages of 40 and 89 years and without a history of liver disease or any smoking- or alcohol-related condition, including ischemic heart disease, lung cancer, peptic ulcer, or pancreatitis. Serum HBsAg status was assessed by RPHA, and anti-HCV status by first-generation EIA. All subjects were interviewed in person to obtain information on use of tobacco and alcohol and history of blood transfusion. Among HBsAg-negative subjects, the OR for anti-HCV positivity was 92.4 (95% CI = 33.8 to 252) after adjustment for age, sex, tobacco smoking, alcohol drinking, and history of blood transfusion.

Okuno *et al.* (1994) studied 186 patients (168 men, 18 women) newly diagnosed with hepatocellular carcinoma at the Guangxi Medical University Affiliated Hospital, in Nanning City, Southern Guangxi, between January 1991 and September 1992. Controls were 48 apparently healthy workers (30 men, 18 women) given a routine physical examination at the same hospital in August 1992. HBsAg status was assessed by RPHA, and anti-HCV status by second-generation EIA. Ten (5.4%) hepatocellular carcinoma patients tested positive for anti-HCV antibodies, versus no controls. Based on tabular data, the lower limit of the 95% CI of the crude OR was 0.6.

A population-based cohort of 9,775 men aged 30 to 85 years from six townships in Taiwan was accrued between September 1984 and February 1986 (Chang *et al.* 1994). At baseline, a structured in-person interview and a blood specimen were obtained from each subject. The interview solicited information on history of tobacco and alcohol use, usual

dietary habits, and family and personal history of liver disease. The cohort was actively followed on an annual basis until March 1992. Chang *et al.* (1994) conducted a nested case-control analysis on 38 men newly diagnosed with primary liver cancer and 152 controls within the cohort. Control subjects were matched by age (within one year), township of residence, and date of recruitment to the index cases. Serum HBsAg status at baseline was determined by RPHA and samples testing negative were rechecked by RIA. Anti-HCV status was determined by second-generation EIA. The matched OR for anti-HCV positivity was 88.2 (95% CI = 5.2 to 1,509) after adjustment for HBsAg status, vegetable intake, and personal history of chronic liver disease. Among HBsAg-negative subjects, the matched OR for anti-HCV positivity was 34.0 (95% CI = 3.5 to 327.8).

Park *et al.* (1995) studied 540 patients (433 men, 107 women) with hepatocellular carcinoma admitted to the Kosin University Hospital, in Pusan, Korea, between July 1992 and February 1994. Controls were 808 apparently healthy residents of Pusan City (431 men, 377 women) given a routine physical examination at the same hospital between September 1992 and October 1993. All control subjects were free of biochemical or clinical features of liver disease at enrollment. HBsAg status was tested by RIA, anti-HCV status by second-generation EIA, and HCV RNA by nested RT-PCR. The OR for anti-HCV positivity with adjustment for HBsAg status was 23.9 (95% CI = 17.4 to 32.9).

A population-based cohort of 18,244 Chinese men, aged 45 to 64 years, in Shanghai were recruited between January 1986 and September 1989. An in-person interview was conducted and blood and urine specimens were collected from each participant at baseline. Yuan *et al.* (1995) studied 76 incident cases of hepatocellular carcinoma within the cohort and 410 control cohort subjects individually matched to the hepatocellular carcinoma subjects by age (within one year), time of blood sample collection (within one month), and neighborhood of residence. Serum HBsAg was measured by RIA, and anti-HCV status by second-generation EIA. Only one hepatocellular carcinoma patient and one control subject tested positive for anti-HCV antibodies (matched OR = 5.0; 95% CI = 0.3 to 79.9).

Sun *et al.* (1996) conducted a population-based case-control study in seven townships in Taiwan involving 58 incident cases of hepatocellular carcinoma (51 in men, 7 in women) and 225 controls matched to the index cases by age (within five years), gender, and township of residence. Second-generation EIAs were used to assess HBsAg and anti-HCV status and RT-PCR to assess HCV RNA. The matched OR for anti-HCV positivity with adjustment for HBsAg was 8.8 (95% CI = 1.8 to 43.0). The comparable OR for HCV RNA positivity was 6.2 (95% CI = 1.4 to 26.6).

Tsai *et al.* (1996) studied 361 patients (303 men, 58 women) newly diagnosed with hepatocellular carcinoma and consecutively admitted to the Kaohsiung Medical College Hospital, in Taiwan, between January 1991 and December 1993. Controls were apparently healthy subjects who entered the same hospital for a physical check-up during the study period; they were individually matched (one control per case) to the index cases by age (within five years) and gender. All subjects were tested for HBsAg, hepatitis Be antigen (HBeAg), and antibodies to HBeAg (anti-HBe) by RIA, and anti-HCV antibodies

by second-generation EIA. The matched OR for anti-HCV positivity after adjustment for HBsAg and HBeAg status was 59.3 (95% CI = 13.6 to 258.4).

Shin *et al.* (1996) conducted a hospital-based case-control study in Pusan, Korea, between August 1990 and August 1993. The 203 case subjects (159 men, 44 women) were newly diagnosed hepatocellular carcinoma patients admitted consecutively to the Inje University Pusan Paik Hospital. Two groups of control subjects were individually matched to the index cases by age (within four years) and sex; these were 203 apparently healthy subjects who entered the same hospital for a routine checkup and 203 hospital inpatients free of cancer or liver disease. Subjects were tested for HBsAg, anti-HBc, and anti-HBs by RIA and anti-HCV by second-generation EIA. A structured questionnaire was used to administer an in-person interview to each subject to obtain information on medical history, family history of cancer and liver disease, smoking and drinking habits, and occupational history. The two control groups yielded similar results and were combined in the reporting of data. The matched OR for anti-HCV positivity after adjustment for HBsAg, *Clonorchis sinensis* in the stool, history of blood transfusion, history of acute hepatitis, history of liver fluke, alcohol drinking, and tobacco smoking was 30.3 (95% CI = 6.1 to 150.6).

Tanaka *et al.* (1996) studied 91 patients (73 men, 18 women) newly diagnosed with hepatocellular carcinoma, aged 40 to 69 years and residents of Fukuoka or Saga Prefecture, who were admitted to the Kyushu University Hospital between December 1985 and June 1989. Controls were 410 residents of Fukuoka City (291 men, 119 women), aged 40 to 69 years, who had a physical examination at a public health center located near the Kyushu University Hospital between January 1986 and July 1989. Control subjects were frequency-matched to the cases by age and had no known history of liver disease. Serum HBsAg status was determined by RPHA, anti-HCV status by second-generation immunoradiometric assay (IRMA), with confirmation by RIBA, and HCV RNA by nested RT-PCR. The sex- and age-adjusted OR for anti-HCV positivity was 53.7 (95% CI = 27.1 to 106.2). Among HBsAg-negative subjects, the comparable OR was 339.6 (95% CI = 96.5 to 1,195.8).

Yu *et al.* (1997b) conducted a case-control study in four areas of southeastern China with a relatively high incidence of hepatocellular carcinoma. A total of 359 hepatocellular carcinoma patients and an equal number of controls individually matched by age (within five years), sex, and location were enrolled; the number of cases in each location ranged from 71 to 100. How the cases and controls were identified and selected was not reported. Serum HBsAg and anti-HCV status were tested with Chinese-manufactured EIA kits; it was unclear whether the anti-HCV assay was first or second generation. The matched OR for anti-HCV positivity was 3.3 (95% CI = 2.0 to 5.6). Among HBsAg-negative subjects, the comparable OR was 2.4 (95% CI = 0.2 to 4.8).

3.3.1.2 Africa

Bile *et al.* (1993) conducted a hospital-based case-control study in Mogadishu, Somalia, in 1989, involving 62 patients with chronic liver disease (49 hepatocellular carcinoma, 13 other liver disease) admitted to two main referral centers. For each case, a hospital control patient, matched for age (within five years) and gender was selected [no further

details on control selection were given]. Serum HBsAg status was assessed by RIA, and anti-HCV status by second-generation EIA. All reported data referred to the 62 chronic liver disease patients and their matched controls; therefore, risk for hepatocellular carcinoma alone cannot be estimated from the published paper. The matched OR for anti-HCV positivity was 9.8 (95% CI = 3.2 to 30.4). The comparable OR in HBsAg-negative subjects was 10.8 (95% CI = 3.0 to 38.6).

Cenac *et al.* (1995) studied 26 Sahelian African hepatocellular carcinoma patients (19 men, 7 women) who were admitted to the Hospital National, in Niamey, Niger, between June 1983 and June 1985. The comparison group consisted of 47 Sahelian African patients (24 men, 23 women) admitted to the Department of Internal Medicine in the same hospital during the study period who had no history of liver disease. Serum HBsAg was assessed by RIA, and anti-HCV status by a second-generation EIA. Anti-HCV positivity rates were 23.1% in cases and 6.4% in controls ($P < 0.05$). Based on tabular data, the crude OR for anti-HCV positivity was calculated to be 4.4 (95% CI = 0.8 to 21.7).

Kew *et al.* (1997) studied 231 black South African patients with hepatocellular carcinoma and controls admitted to four Johannesburg hospitals who were individually matched by age (within two years), sex, race, background (rural, urban, or rural-urban), hospital, and medical versus surgical ward). Serum HBsAg was assessed by RIA, anti-HCV status by second- or third-generation EIA, and HCV RNA by nested RT-PCR. The matched OR for anti-HCV positivity was 6.1 (95% CI = 2.8 to 13.7). Among HBsAg-negative subjects, the comparable OR was 6.6 (95% CI = 2.7 to 15.7).

3.3.1.3 Americas

A cohort of 5,924 Japanese-American men in Hawaii was accrued between 1967 and 1970 and followed for cancer occurrence until 1992 (in the Japan-Hawaii Cancer Study). Nomura *et al.* (1996) conducted a nested case-control analysis of 24 incident cases of hepatocellular carcinoma and 72 age-matched cohort controls. Serum HBsAg status was determined by RIA. All subjects were first tested for anti-HCV by first-generation EIA; samples with positive results were retested by RIBA. None of the cases and only one control tested positive for anti-HCV antibodies.

Yu *et al.* (1997a) conducted a population-based case-control study among non-Asians of Los Angeles County, California, between the ages of 18 and 74 years. The study included 111 patients newly diagnosed with hepatocellular carcinoma and 128 community controls. Subjects were assessed for HBsAg, anti-HBc, and anti-HBs by RIA, and anti-HCV by second- generation EIA, with confirmation by RIBA. The age- and sex-adjusted OR for anti-HCV positivity was 12.6 (95% CI = 4.7 to 33.6). The comparable OR among subjects who tested negative for all serological markers of HBV infection was 6.5 (95% CI = 2.1 to 19.9).

3.3.1.4 Europe

Hadziyannis *et al.* (1995) conducted a hospital-based case-control study in Athens, Greece, involving 65 incident cases of hepatocellular carcinoma and two groups of hospital controls individually matched to the index cases by age (within five years) and

gender (65 metastatic liver cancer patients and 65 patients hospitalized for eye, ear, nose, or throat conditions). Study subjects were tested for HBsAg, anti-HBc, and anti-HBs by EIA, and anti-HCV by second-generation EIA with confirmation by RIBA. A structured questionnaire was used to interview all subjects in person to obtain information on medical history, diet, and other lifestyle factors. Combining the two groups of control subjects, the matched OR for anti-HCV positivity after adjustment for HBsAg was 7.7 (95% CI = 1.7 to 35.1). Further adjustment for potential confounders, including history of blood transfusion, cigarette smoking, alcohol drinking, coffee drinking, and history of diabetes, did not materially change the HCV-hepatocellular carcinoma association.

A hospital-based case-control study was conducted in two hospitals in Goteborg, Sweden, from 1984 to 1991 (Kaczynski *et al.* 1996). The study compared 73 hepatocellular carcinoma patients (55 men, 18 women) with 32 patients with other cancers (N = 21) or benign liver disease (N = 11), who served as control subjects. Serum HBsAg was tested by RIA, and anti-HCV status by third-generation EIA, with confirmation by RIBA. Anti-HCV antibodies were found in of 7 of 64 hepatocellular carcinoma patients (11%) versus 1 of 31 controls (3%). Based on tabular data, the crude OR for anti-HCV positivity was 3.7 (95% CI = 0.5 to 84.5).

Donato *et al.* (1997) studied 172 newly diagnosed hepatocellular carcinoma patients in the two major hospitals in the province of Brescia, in northern Italy. Other hospital patients without a history of liver disease or cancer served as the control group. They were frequency-matched to the cases by age (five-year age groups), sex, and date and hospital of admission. Subjects were tested for HBsAg by EIA, anti-HCV by third-generation EIA, with confirmation by RIBA, and HCV RNA by nested RT-PCR. Information on alcohol intake was obtained through in-person interviews. The OR for anti-HCV positivity without HCV RNA, after adjustment for age, sex, residence, HBsAg, and heavy alcohol intake, was 1.5 (95% CI = 0.4 to 6.0). The comparable OR for concurrent anti-HCV and HCV RNA positivity was 23.2 (95% CI = 11.8 to 45.7).

3.3.1.5 Meta-analysis

Table 3-2 shows the results of the meta-analysis. Studies employing the first-generation anti-HCV assay found considerably higher ORs for anti-HCV positivity than those using later-generation assays (19.1 versus 8.2). On the basis of these findings, the authors conducted comparisons by geographical area (high- versus low-risk regions) and type of control subjects (hospital versus community) only among studies employing the second- or third-generation anti-HCV assays. The summary OR from studies employing community controls was slightly higher than that derived from studies using hospital controls (9.0 versus 6.8). A stronger association between HCV infection and hepatocellular carcinoma risk was noted in areas with low to intermediate incidence rates of hepatocellular carcinoma (OR = 16.8) than in Asia and Africa, where both HBV infection and hepatocellular carcinoma are endemic (OR = 6.2).

3.3.2 Other case-control studies

Additional case-control studies are discussed below and are summarized in Table 3-3.

3.3.2.1 Asia

Yuan *et al.* (1996) studied 39 patients with hepatocellular carcinoma admitted to the Affiliated Hospital of the Guangxi Medical College, in Nanning City, Southern Guangxi, China, during 1982. One control group consisted of 41 patients with age and sex distributions similar to those of the cases and admitted to the same hospital during the study period for reasons other than liver disease. A second control group consisted of 100 healthy subjects drawn from among residents of the hospital's catchment area. Subjects were tested for HBsAg, anti-HBc, and anti-HBs by RIA, and anti-HCV by second-generation EIA, with confirmation by RIBA. None of the 39 hepatocellular carcinoma patients (upper 95% CI = 6.1%) and only 1of the 141 control subjects (0.7%; upper 95% CI = 3.2%) tested positive for anti-HCV.

Okada *et al.* (1998) conducted a hospital-based case-control study of hepatocellular carcinoma among patients with chronic liver disease at the National Cancer Center Hospital, in Tokyo, Japan, between January 1992 and December 1993. The study included 141 consecutive patients (110 men, 31 women) with hepatocellular carcinoma and underlying chronic liver disease, between the ages of 25 and 81 years. Controls were 151 consecutive patients (96 men, 55 women) with chronic liver disease but no evidence of hepatocellular carcinoma (aged 25 to 79 years) who were admitted to the same hospital during the study period. All subjects were tested for HBsAg by RPHA, anti-HBs by passive hemagglutination, anti-HBc by EIA, and anti-HCV by second-generation EIA. The OR for anti-HCV positivity after adjustment for age, sex, HBsAg status, and anti-HBc status was 2.3 (95% CI = 1.1 to 4.9). Among HBsAg-negative subjects, the age- and sex-adjusted OR for anti-HCV positivity was 4.0 (95% CI = 1.7 to 9.4).

Zhang *et al.* (1998) studied 152 patients (136 men, 16 women) with hepatocellular carcinoma admitted to four hospitals in Henan Province, China, between January 1994 and October 1995. Controls were 115 (99 men, 16 women) patients admitted to the same hospitals during the study period, with ages similar to those of the hepatocellular carcinoma patients and judged to be free of liver disease. Study subjects were tested for HBsAg and anti-HBs by RIA, anti-HBc and anti-HCV by EIA (unknown generation), HBV DNA by PCR, and HCV RNA by RT-PCR. A structured questionnaire was used to conduct in-person interviews to solicit information on medical history, history of cigarette and alcohol use, usual diet, history of blood transfusion, and family history of cancer and liver disease. Anti-HCV positivity rates in cases and controls were 11.2% and 3.5%, respectively ($P < 0.05$). Based on tabular data, the crude OR was 3.5 (95% CI = 1.1 to 11.6). Among HBsAg-negative subjects, 5 case and 4 control subjects were anti-HCV positive (OR = 2.6; 95% CI = 0.6 to 12.0).

3.3.2.2 Americas

Yuan *et al.* (1999) reported on the latest results of the population-based Los Angeles Study, which was included in the meta-analysis of Donato *et al.* (1998) (see Section 3.3.1). The latest findings were based on 144 non-Asian patients with hepatocellular carcinoma and 252 community controls of similar age, gender, and race. Samples were tested for HBsAg by RIA, anti-HBsAg and anti-HBcAg by EIA, anti-HCV by first- or second-generation EIA, and HCV RNA by RT-PCR. The age- and sex-adjusted OR for

anti-HCV positivity was 18.8 (95% CI = 7.1 to 49.7). Concurrent positivity for anti-HCV antibodies and HCV RNA carried a much higher risk of cancer than did anti-HCV positivity in the absence of HCV RNA. The OR for concurrent anti-HCV and HCV RNA positiviy carried a lower 95% confidence limit of 23.1 (no control subjects tested positive for both anti-HCV and HCV RNA, so no finite OR estimate can be calculated). For anti-HCV positivity in the absence of HCV RNA, the OR was 4.5 (95% CI = 1.5 to 13.9). Among HBsAg-negative subjects, the age- and sex-adjusted OR for anti-HCV positivity was 20.1 (95% CI = 6.6 to 61.2).

3.3.2.3 Europe

Peters *et al.* (1994) studied 86 patients (74 men [also reported as 71 men in one table], 14 women) with hepatocellular carcinoma and underlying liver cirrhosis who were seen at the University Hospital of Mainz, Germany, between 1986 and 1993. Controls were patients who were seen at the same hospital during the study period, individually matched (by age within 5 years and sex), who had liver cirrhosis but no evidence of hepatocellular carcinoma. All subjects were tested for HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe, and anti-delta antibody by RIA. Subjects admitted to the hospital before January 1991 were tested for anti-HCV by first-generation EIA, and those admitted subsequently were tested for anti-HCV by second-generation EIA. Information on cigarette and alcohol use was abstracted from patient charts. The matched OR for anti-HCV positivity was 2.1 (95% CI = 0.8 to 5.6). Among HBV-positive subjects, the comparable OR after adjustment for tobacco and alcohol intake was 6.4 (95% CI = 1.1 to 35.2).

Goritsas *et al.* (1995) studied 51 consecutively admitted patients (48 men, 3 women) with hepatocellular carcinoma at the Patras University Hospital, in Patras, Greece, between October 1989 and October 1992. Controls were patients seen at the same hospital during the study period, individually matched (by sex and age within five years), who did not have a history of liver disease or cancer. Study subjects were tested for HBsAg, anti-HBs, and anti-HBc by EIA, and anti-HCV by second-generation EIA with confirmation by RIBA. Information on alcohol and tobacco use was obtained through in-person interviews. The matched OR for anti-HCV positivity after adjustment for HBsAg status, heavy alcohol intake, and tobacco smoking was 1.9 (95% CI = 1.1 to 3.3).

Tagger *et al.* (1999) reported on the latest results of the ongoing hospital-based Brescia study, which was included in the meta-analysis of Donato *et al.* (1998) (see Section 3.3.1). The latest findings were based on 305 cases of hepatocellular carcinoma and 610 hospital controls. HBV markers (HBsAg, anti-HBs, and anti-HBc) were assessed by EIA, anti-HCV by third-generation EIA, with confirmation by RIBA, and HCV genotypes by nested RT-PCR. The presence of anti-HCV without HCV RNA was not significantly related to hepatocellular carcinoma risk. After adjustment for age, sex, alcohol intake, and HBV infection, the OR for anti-HCV without HCV RNA was 1.5 (95% CI = 0.5 to 4.5). The comparable OR for concurrent positivity for anti-HCV antibodies and HCV RNA was 26.3 (95% CI = 15.8 to 44.0).

Chiesa *et al.* (2000) studied 142 patients (116 men, 26 women) with hepatocellular carcinoma in the presence of liver cirrhosis and 21 patients (19 men, 2 women) with hepatocellular carcinoma without cirrhosis who were admitted to two main hospitals in

Brescia, Italy, between 1995 and 1997. Controls were 610 patients admitted to the same hospitals during the study period who were free of liver disease. Serum HBsAg status was assessed by EIA, HBV DNA by PCR, anti-HCV by third-generation EIA, and HCV RNA by RT-PCR. The age- and sex-adjusted OR for concurrent anti-HCV and HCV RNA positivity in the presence of cirrhosis was 33.5 (95% CI = 17.7 to 63.4). The comparable OR in the absence of cirrhosis was 19.7 (95% CI = 6.0 to 64.8).

Kuper *et al.* (2000) studied 333 patients newly diagnosed with hepatocellular carcinoma and treated at three teaching hospitals in Athens, Greece, from January 1995 to December 1998. Two control groups admitted to the same hospitals during the study period and with age and sex distributions similar to those of the hepatocellular carcinoma patients were accrued. The first control group consisted of 272 patients with metastatic liver cancer, and the second of 360 patients hospitalized for eye, ear, nose, or throat conditions. All subjects were tested for HBsAg and anti-HCV status by third-generation EIA. Combining the two control groups, the OR for anti-HCV positivity after adjustment for age, sex, schooling, and HBsAg status was 23.2 (95% CI = 11.4 to 47.3). Among HBsAg-negative subjects, the comparable OR was 32.3 (95% CI = 15.0 to 69.4).

3.3.3 Cohort studies

Table 3-4 summarizes the results of cohort studies.

Kato *et al.* (1994) assembled a cohort of 401 patients (273 men, 128 women) with cirrhosis from April 1977 to March 1992 in the city of Nagasaki, Japan, who had no history or evidence of hepatocellular carcinoma at enrollment. Baseline blood samples collected from subjects were tested for HBsAg by RIA and anti-HCV by second-generation EIA or RIA. By March 1993 (mean follow-up time = 4.4 years), 127 cases of hepatocellular carcinoma had developed within the cohort. The five-year cumulative rate of hepatocellular carcinoma among subjects negative for both HBsAg and anti-HCV antibodies was 12.4%. The comparable figure in subjects positive only for anti-HCV antibodies was 36.9%, and the difference between the two rates was statistically significant ($P < 0.01$).

Tsai *et al.* (1997) followed a cohort of 400 patients (290 men, 110 women) with non-alcoholic cirrhosis at the Kaohsiung Medical College Hospital from January 1989 to December 1994. After 1,185 person-years of follow-up, 80 new cases of hepatocellular carcinoma had occurred in this cohort. Baseline serum samples collected from study subjects were tested for HBsAg by RIA and for anti-HCV by second-generation EIA. The incidence of hepatocellular carcinoma among subjects negative for HBsAg and anti-HCV antibodies at baseline was 2.0%. The comparable figure for subjects who tested positive at baseline only for anti-HCV antibodies was 7.0%. The relative risk of hepatocellular carcinoma for anti-HCV positivity alone was 3.7 (95% CI = 1.1 to 13.1) after adjustment for age, sex, and other potential confounders.

A multinational cohort of 1,438 patients with cirrhosis was assembled between 1978 and 1988 (Naoumov *et al.* 1997). The median follow-up time was 5 years (range = 1 to 16 years). A nested case-control analysis was conducted on 72 cohort members (60 men, 12 women) newly diagnosed with hepatocellular carcinoma and an equal number of cohort

controls individually matched by age, sex, country of birth, HBsAg status, duration of follow-up, and etiology of cirrhosis. Subjects were tested for HBsAg, anti-HBs, anti-HBc, and anti-HCV by EIA, and HCV RNA by RT-PCR. The matched OR for HCV RNA positivity at baseline was 2.4 (95% CI = 1.2 to 5.0). The comparable OR for anti-HCV positivity was 2.1 (95% CI = 1.0 to 4.4).

Ikeda *et al.* (1998) followed a cohort of 2,215 patients (1,544 men, 671 women) with chronic viral hepatitis diagnosed at the Toranomon Hospital in Tokyo, Japan, between January 1980 and August 1995. Within the cohort, 89 cases of hepatocellular carcinoma developed after a median follow-up period of 4.1 years (range = 0.1 to 16.3 years). Serum HBsAg and anti-HCV status at baseline were established by RIA and second-generation EIA, respectively. Anti-HCV positivity was a significant risk factor for development of hepatocellular carcinoma among cohort members ($P = 0.01$).

Mori *et al.* (2000) recruited a population-based cohort of 3,059 residents (981 men, 2,078 women) of Saga Prefecture, Japan, aged 30 years or older, during June 1992. At baseline, a blood specimen and a written questionnaire pertaining to medical and family histories and use of tobacco and alcohol were collected from each participant. Baseline serum samples were tested for HBsAg by RPHA and anti-HCV by second-generation EIA. By March 1997 (median follow-up time = 4.8 years), 22 cohort members (14 men, 8 women) had developed hepatocellular carcinoma. The age- and sex-adjusted relative risk of hepatocellular carcinoma for high-titer anti-HCV positivity at baseline was 40.4 (95% CI = 11.7 to 139.2).

3.3.4 HCV genotype

On the basis of nucleotide sequence homology, several HCV genotypes have been identified (see Sections 1.1.2, 2.2.2.3, and 2.3.1.3). A number of studies have examined the possibility that the various HCV genotypes may differ in their carcinogenic potencies. Although the results are not entirely consistent, the overall evidence supports the hypothesis that HCV genotype 1b is more strongly associated with hepatocellular carcinoma than other HCV genotypes. Studies of specific HCV genotypes and hepatocellular carcinoma are discussed below, and the results are summarized in Table 3-5.

In Japan, Yotsuyanagi *et al.* (1995) studied 203 consecutive patients with chronic HCV infection who were seen at the University of Tokyo Hospital between 1991 and 1993. All patients tested positive for anti-HCV antibodies by second-generation EIA and positive for HCV RNA by RT-PCR. All patients persistently tested HBsAg-negative. HCV genotyping was performed by RT-PCR. At the time of this study, 72 of the 203 patients had developed hepatocellular carcinoma. Rates of HCV genotype 1b among the 72 hepatocellular carcinoma patients and the remaining 131 cancer-free subjects were 79% and 77%, respectively ($P > 0.05$, test of difference in rates). Based on tabular data, the crude OR for 1b genotype was 1.1 (95% CI = 0.5 to 23).

In Japan, Takada *et al.* (1996) compared rates of 1b genotype among three groups of HCV-positive liver disease patients attending 14 hospitals: 1,922 with chronic hepatitis, 356 with liver cirrhosis, and 426 with hepatocellular carcinoma. HCV subtypes

(genotypes) were determined by PCR with type-specific primers or slot-blot hybridization with type-specific probes. Rates of HCV genotype 1b in the three groups of patients were 71%, 80%, and 81%, respectively. The difference in rates between chronic hepatitis patients and patients with either liver cirrhosis or hepatocellular carcinoma was statistically significant ($P < 0.05$). Based on tabular data, the crude OR for HCV genotype 1b relative to chronic hepatitis patients was 1.7 (95% CI = 1.3 to 2.2).

Silini *et al.* (1996) examined three groups of anti-HCV-positive liver-disease patients seen at two hospitals in northern Italy between 1991 and 1994: (1) 593 patients with chronic hepatitis, (2) 219 patients with cirrhosis but no evidence of hepatocellular carcinoma, and (3) 166 patients with cirrhosis and hepatocellular carcinoma. All patients were tested for anti-HCV antibodies by second-generation EIA, with confirmation by RIBA. RT-PCR was used to detect HCV RNA and for HCV genotyping. The rate of HCV genotype 1b in hepatocellular carcinoma patients was 72%, which was significantly higher than that in patients with cirrhosis only (57%) or chronic hepatitis only (46%). The age-, sex- and Child's class-adjusted OR for hepatocellular carcinoma in genotype 1b cirrhosis patients was 2.0 (95% CI = 1.3 to 3.1).

Hatzakis *et al.* (1996) compared three groups of anti-HCV-positive liver-disease patients seen at two hospitals in Athens, Greece: (1) 17 patients with hepatocellular carcinoma, (2) 24 patients with chronic hepatitis and cirrhosis, and (3) 101 patients with chronic hepatitis in the absence of cirrhosis. All patients were tested for anti-HCV by third-generation EIA, and HCV RNA by RT-PCR. HCV genotyping was determined by a reverse hybridization assay. Rates of HCV genotype 1b in the three groups of patients were 70.6%, 69.6%, and 26.4%, respectively. Relative to cirrhosis-negative chronic hepatitis patients, the age- and sex-adjusted OR for 1b genotype was 8.3 (95% CI = 1.2 to 57.5).

In Korea, Lee *et al.* (1996) examined sera from 30 blood donors, 30 patients on maintenance hemodialysis, 33 patients with chronic hepatitis, 15 patients with liver cirrhosis, and 30 patients with hepatocellular carcinoma, all of whom were anti-HCV positive. No information was given regarding the source of the patients. Second-generation EIA was used for anti-HCV testing, and RT-PCR for testing HCV RNA and genotyping HCV. The rates of HCV genotype 1b were 60% in hepatocellular carcinoma patients, 60% liver cirrhosis patients, and 64% in chronic hepatitis patients. Rates were highest in hemodialysis patients (87%) and blood donors (80%). Based on tabular data, the crude OR for 1b genotype, with chronic HCV patients with or without cirrhosis as controls, was 0.9 (95% CI = 0.3 to 2.3).

In Spain, Lopez-Labrador *et al.* (1997) studied three groups of patients with HCV-related liver disease seen at a university hospital in Barcelona: 243 with asymptomatic chronic hepatitis, 59 with decompensated cirrhosis but no evidence of hepatocellular carcinoma, and 112 with liver cirrhosis and hepatocellular carcinoma. Anti-HCV was tested by third-generation EIA, and HCV RNA by nested RT-PCR. HCV genotyping was performed by RFLP analysis. Rates of HCV genotype 1b were highest in patients with hepatocellular carcinoma (97%), intermediate in patients with cirrhosis (93%), and lowest in patients with asymptomatic chronic hepatitis (72%); the difference was statistically significant (*P*

< 0.001). Based on tabular data, the crude OR for 1b genotype relative to patients with asymptomatic chronic hepatitis was 13.8 (95% CI = 4.5 to 52.9).

In Italy, Bruno *et al.* (1997) followed 163 consecutive anti-HCV-positive patients with cirrhosis, enrolled between January 1989 and December 1990, for the occurrence of hepatocellular carcinoma. Anti-HCV was determined by second-generation EIA, HCV RNA by nested RT-PCR, and HCV genotype by PCR. After a median follow-up period of 68 months, hepatocellular carcinoma had developed in 22 cirrhotic patients. The age- and sex-adjusted relative risk for genotype 1b, after adjustment for alcohol abuse and interferon treatment, was 6.1 (95% CI = 1.8 to 21.4).

The study of Naoumov *et al.* (1997) is described in Section 3.3.3, above. This was a nested case-control study within a multinational cohort of patients with cirrhosis. Rates of HCV genotype 1b in the 30 hepatocellular carcinoma patients and 17 controls positive for HCV RNA were 50% and 59%, respectively. The two rates did not differ signficantly. Based on tabular data, the crude OR for 1b genotype was 0.7 (95% CI = 0.2 to 2.6).

3.3.5 Combined effect of HBV and HCV

As stated in Section 3.3, 11 of the 32 studies summarized in the meta-analysis by Donato *et al.* (1998) were part of the IARC evaluation (IARC 1994) and are not individually reviewed in this report. Another study was excluded because the abstract (Yang *et al.* 1996) never resulted in a full-length publication. The study design and findings pertaining to the association between HCV and hepatocellular carcinoma for each of the remaining 20 studies are briefly described above, in Section 3.3.1. Table 3-6 shows the stratified effects of HBV and HCV infections on hepatocellular carcinoma risk observed in each of the 20 studies.

Table 3-7 shows the meta-analysis results of Donato *et al.* (1998), derived from 4,560 cases of hepatocellular carcinoma and 6,988 control subjects. The summary OR for hepatocellular carcinoma risk in individuals positive only for HBsAg was similar to that for those positive only for anti-HCV or HCV RNA; both were approximately 20. A synergistic effect (greater than the sum of the separate effects of each infection) was noted for coinfection with HBV and HCV; the summary OR for the combined presence of HBsAg and anti-HCV antibodies or HCV RNA was 135. Studies employing community controls tended to yield larger estimates of the interaction effect than did studies with hospital controls. [One should be cautious in interpreting the magnitude of OR differences for HBV and HCV coinfection across subgroups of studies (last column of Table 3-7). Because the number of coinfected subjects in each study was very small (see Table 3-6), the subgroup risk estimates were highly unstable.] In Asia and Africa, where the role of HCV in hepatocellular carcinoma development is relatively minor, the summary OR associated with HCV infection alone (11.5) is less than half the summary OR (31.2) derived from studies in Japan, North America, and Europe, low- to intermediate-risk regions where there is growing evidence that HCV infection is largely responsible for the increasing incidence of hepatocellular carcinoma (Yu *et al.* 2000, El-Serag and Mason 2000).

Table 3-8 shows the results of studies published after the IARC review but not part of the Donato *et al.* (1998) meta-analysis (described in Sections 3.3.2 and 3.3.3, above). Four of the nine case-control studies reported on the interactive effects of HBV and HCV infections on risk of hepatocellular carcinoma. Three of these four studies demonstrated a synergistic effect of HBV and HCV coinfection on risk that was consistent with the meta-analysis results of Donato *et al.* (1998). All four studies suffer from relatively small numbers of cases and controls in the HBV-HCV coinfection category. None of the recent cohort studies (Table 3-4) examined the effect of coinfection with HBV and HCV on hepatocellular carcinoma risk.

3.3.6 Modifying effects of nonviral cofactors on the HCV-hepatocellular carcinoma association

A case-control study (Tagger *et al.* 1999) provided some evidence that heavy alcohol intake may enhance the carcinogenic effect of HCV infection; the combined effect of both exposures appeared to be greater than the sum of the separate effects of each exposure. This study is described in Section 3.3.2.3. Tagger *et al.* (1999) reported an OR of 26.1 (95% CI = 12.6 to 54.0) for HCV RNA positivity and light drinking (0 to 40 g of ethanol per day). The comparable OR for HCV RNA negativity and heavy drinking (> 80 g of ethanol per day) was 7.3 (95% CI = 4.0 to 13.1), and the OR for HCV RNA positivity and heavy drinking was 126 (95% CI = 42.8 to 373) (Table 3-9).

3.4 Studies of B-cell lymphoma

Since the IARC review conducted in July 1993 (IARC 1994), a number of reports have linked HCV infection to increased risk of B-cell lymphoma. These studies are individually reviewed in this section and summarized in Table 3-10.

3.4.1 Case-control studies

3.4.1.1 Asia

In Japan, Izumi *et al.* (1997) studied 50 patients with B-cell malignancy (25 non-Hodgkin’s lymphoma, 4 Waldenstrom’s macroglobulinemia, 21 multiple myeloma) and 18 patients with non-B-cell lymphoma who served as controls. No information was given on the source of these patients or their age and sex distributions. All subjects were tested for anti-HCV by second-generation EIA and HCV RNA by RT-PCR. The rate of anti-HCV positivity in patients with B-cell malignancy was 16% (16% in B-cell lymphoma, 25% in Waldenstrom’s macroglobulinemia, and 14% in multiple myeloma). None of the control patients were anti-HCV positive, comparable to the rate of 1% among healthy blood donors in Japan. Based on tabular data, the lower limit of the 95% CI of the crude OR for B-cell malignancy was 0.8.

In Japan, Mizorogi *et al.* (2000) studied 100 patients with B-cell lymphoma, 25 patients with non-B-cell lymphoma, and 516 patients with miscellaneous diseases other than liver disease or B-cell lymphoproliferative disorders. All subjects were patients at the Daisan Hospital, in Tokyo, between January 1993 and December 1998. Anti-HCV testing was conducted using a second-generation EIA. The rate of anti-HCV positivity in patients with B-cell lymphoma was 17%. The comparable rates in patients with non-B-cell

lymphoma and controls were 0% and 6.6%, respectively. Based on tabular data, the crude OR for anti-HCV positivity was 2.9 (95% CI = 1.5 to 5.4).

3.4.1.2 Americas

Zuckerman *et al.* (1997) studied 120 consecutive patients with B-cell lymphoma seen at the Los Angeles County-University of Southern California Medical Center between October 1994 and May 1996. Two groups of controls were assembled: (1) 154 patients with hematologic malignancies other than B-cell lymphoma, seen at the same hospital during the study period, and (2) 114 general medicine clinic patients seen at the same hospital during the study period, without hematologic malignancies. Subjects were tested for anti-HCV by second-generation EIA and HCV RNA by RT-PCR. Infection with HCV was detected in 22% of B-cell lymphoma patients, but only 4.5% and 5% of the two control groups ($P < 0.001$, test of difference in rates). Based on tabular data (combining the two control groups), the crude OR for HCV infection was 5.4 (95% CI = 2.6 to 11.5).

Collier *et al.* (1999) studied 100 patients with B-cell lymphoma (10 high grade, 46 intermediate grade, and 44 low grade; 54 men, 46 women) who were seen at the Princess Margaret Hospital, in Toronto, Canada, between February and May 1997. Control subjects were 100 patients (59 men, 41 women) with gastrointestinal malignancies, of ages similar to those of the B-cell lymphoma patients, who were seen at the same hospital over the study period. All subjects were tested for anti-HCV by third-generation EIA and HCV RNA by PCR. All cases and controls tested negative for anti-HCV antibodies and HCV RNA.

3.4.1.3 Europe

In Italy, Ferri *et al.* (1994) studied 50 unselected patients (26 men, 24 women) with B-cell lymphoma; 30 Hodgkin's disease patients and 30 healthy subjects of similar ages constituted two comparison groups. No information was given regarding the source of the case and control subjects. Subjects were tested for anti-HCV by second-generation EIA, with confirmation by second-generation RIBA. HCV RNA was determined by nested PCR. Serological markers of HBV infection (HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe) were tested by EIA. Rates of HBV infection were similar among the B-cell lymphoma patients (30%) and the two groups of controls (25% in Hodgkin's disease patients and 22% in healthy subjects). Rates of anti-HCV and HCV RNA positivity among B-cell lymphoma patients were 30% and 32%, respectively. All except two patients were concordant on positivity for anti-HCV and HCV RNA. None of the controls tested positive for HCV RNA. Anti-HCV antibodies were detected in 3% of Hodgkin's disease patients and none of the 30 healthy controls. Based on tabular data (combining the two groups of controls), the crude OR for anti-HCV was 25.3 (95% CI = 3.7 to 533).

Mazzaro *et al.* (1996) studied 199 consecutive non-Hodgkin's lymphoma patients seen at three medical centers in northern Italy. Two groups of controls were obtained: 153 patients with other hematological malignancies, and 6,917 healthy residents of two towns within the catchment area of the three medical centers. Group 1 controls were similar in ages to non-Hodgkin's lymphoma patients. Subjects were tested for anti-HCV by second-generation EIA, with confirmation by RIBA. The overall rate of anti-HCV positivity

among non-Hodgkin's lymphoma patients was 28%, with a higher rate (38%) among those with low-grade lymphomas. The comparable rates in other cancer patients and healthy controls were 3.1% and 2.9%, respectively; both rates were significantly lower than the rate in non-Hodgkin's lymphoma patients. Based on tabular data, the crude OR for anti-HCV positivity versus controls with other hematological malignancies was 11.6 (95% CI = 4.6 to 31.1).

In Italy, Vallisa *et al.* (1999) studied 175 consecutive patients with B-cell lymphoma at the Civil Hospital, in Piacenza, Italy. Controls individually matched by sex and age (within 2 years) were obtained from outpatient clinics (N = 175) and hospital wards (N = 175). Anti-HCV testing was by second-generation EIA, with confirmation by RIBA. The rate of anti-HCV positivity was 37% among B-cell lymphoma patients, but only 9% and 10% among the outpatient and inpatient controls, respectively ($P = 0.0001$, test for difference in rates). Based on tabular data (using all controls), the crude OR for anti-HCV positivity was 5.7 (95% CI = 3.5 to 9.3).

Pioltelli *et al.* (2000) studied 300 consecutive patients (145 men, 155 women) with B-cell lymphoma diagnosed at 11 institutions in Lombardy, Italy, between January 1996 and June 1997. There were three separate control groups: (1) 600 control patients without a history of liver disease, cancer, or autoimmune disease, who had never been treated with corticosteroid or immunosuppressive agents, and who were individually matched by age and sex; (2) 247 consecutive patients (122 men, 125 women) with solid neoplasms, and (3) 122 consecutive patients (14 men, 108 women) with systemic lupus erythematosus and rheumatoid arthritis, who had been treated with corticosteroid or immunosuppressive drugs for at least one year. Subjects were tested for HBsAg by EIA and anti-HCV by third-generation EIA, with confirmation by RIBA. The rate of anti-HCV positivity among B-cell lymphoma patients was 16%. The comparable rates in the three controls groups were 8.5%, 8.5%, and 4.9%, respectively. Versus Group 1 controls, the matched OR for anti-HCV positivity was 2.0 (95% CI = 0.7 to 27.0). However, the rate of HBsAg positivity also was higher in the case than control subjects. The rate among B-cell lymphoma patients was 7.7%, while comparable rates in the three control groups were 0.8%, 3.6%, and 1.6%, respectively. Versus Group 1 controls, the matched OR for HBsAg positivity was 9.9 (95% CI = 3.7 to 26.2).

3.4.2 Cohort studies

Ohsawa *et al.* (1999) accrued a cohort of 2,162 patients (1,328 men, 834 women) with HCV-related chronic liver hepatitis from three hospitals in Osaka, Japan, between 1957 and 1997. Presence of anti-HCV in serum was determined by third-generation EIA, and HCV RNA was detected by nested PCR. After 12,405 person-years of follow-up, four new cases of B-cell lymphoma had developed within the cohort, when the number expected based on sex-, age-, and calendar-year-matched general population rates was 1.9 (RR = 2.1; 95% CI = 0.6 to 5.4) (Table 3-11).

3.5 Summary

An IARC Working Group evaluated the carcinogenic risk to humans of chronic HCV infection in 1993 and concluded that "the agent is carcinogenic to humans" (Group I).

Cohort and case-control studies published since the IARC review, conducted in populations differing by race-ethnicity and geography, have further strengthened the recognized association between chronic HCV infection and development of hepatocellular carcinoma. The recent studies generally used relatively sensitive and specific serological markers to assess chronic HCV infection, and many included information on potential confounders, such as use of alcohol and tobacco. These studies unequivocally show that the strong association between HCV and hepatocellular carcinoma is independent of HBV infection (i.e., the association is clearly present when subjects are confined to individuals who do not carry HBV) and essentially unaltered after adjustment for nonviral risk factors for hepatocellular carcinoma.

A number of recent studies have examined the possibility that the various HCV genotypes may differ in their carcinogenic potencies. Although the results are not entirely consistent, the overall evidence supports the hypothesis that HCV genotype 1b is more strongly associated with hepatocellular carcinoma than are other HCV genotypes.

Several recent studies have specifically examined the effect of the presence of HCV RNA in serum on the risk of hepatocellular carcinoma among anti-HCV-positive subjects. Data are uniform in showing a substantially higher risk when HCV RNA is present.

In the United States, the incidence of hepatocellular carcinoma has increased approximately 70% over the past two decades. Increasing trends in the incidence of hepatocellular carcinoma also have been noted in Japan and Western Europe. There is growing evidence that HCV infection is largely responsible for the secular trends in incidence of hepatocellular carcinoma in these regions of low to intermediate risk of hepatocellular carcinoma (El-Serag and Mason 2000, Yu *et al.* 2000). In contrast, evidence points to a relatively minor role of HCV in the development of hepatocellular carcinoma in regions where it is endemic (Sub-Sahara Africa, East and Southeast Asia) and where early-life infection with HBV is believed to account for 80% or more of the local cases (Yu *et al.* 2000).

Consistent and abundant data support a synergistic effect of HBV and HCV coinfection on risk of hepatocellular carcinoma. There is also evidence that heavy alcohol intake acts as a cofactor to enhance the risk of hepatocellular carcinoma in HCV-infected individuals.

A number of recent case-control studies have linked HCV infection to increased risk of B-cell lymphoma. Many of these studies had relatively small sample sizes, and all were hospital-based. Better-designed, population-based case-control and cohort studies are needed to confirm these preliminary findings.

Table 3-1. Results of individual studies included in the meta-analysis of Donato *et al.* (1998): Odds ratios for hepatocellular carcinoma in subjects testing positive for anti-HCV antibodies versus subjects testing negative

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Fukuda *et al.* 1993 Japan	Hospital-based case-control study Cases: 368 hepatocellular carcinoma (HCC) patients (287 men, 81 women), aged 40 to 69, residents of Fukuoka or Saga prefecture, diagnosed at the Kurume University Hospital between April 1986 and May 1992 Controls: inpatients of two university-affiliated general hospitals in Kurume, judged to be free of chronic hepatitis or hepatic cirrhosis, matched to index cases by age (5-year age groups), sex, residence (prefecture), and time of hospitalization (within 2 months of the case interview) Case-control ratio: 1:1 for men and 1:4 for women	Anti-HCV: 1st-generation EIA (82.8%/7.0%) HBsAg: RPHA or EIA (data from hospital records)	35.0 ($P < 0.05$) – (4.8, –)[a]	Age-matched OR in men only Comparable OR in women

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Cordier *et al.* 1993 Vietnam	Hospital-based case-control study (men only) in Hanoi, Vietnam, 1989–1992 <u>Cases:</u> 152 male HCC patients <u>Controls:</u> 241 male hospital controls of similar ages, admitted to the same hospitals for reasons other than cancer or liver disease	Anti-HCV: 2nd-generation EIA (2.0%/0.8%) HBsAg: 2nd-generation EIA	2.0 (0.3–17.4) 38.1 (2.8–1,443)	OR was age-adjusted Comparable OR for HBsAg(-) subjects

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Pyong *et al.* 1994 Japan	Hospital-based case-control study Cases: 90 Korean patients (68 men, 22 women) newly diagnosed with HCC at the Kyowa Hospital in Osaka, Japan, between January 1989 and December 1992 Controls: 249 Korean patients admitted to the same hospital during the same time period as the cases; aged 40 to 89, without a history of liver disease or any smoking- or alcohol-related conditions, including ischemic heart disease, lung cancer, peptic ulcer, or pancreatitis	Anti-HCV: 1st-generation EIA (74.4%/8.0%) HBsAg: RPHA	92.4 (33.8–252)	OR for HBsAg(-) subjects only; further adjusted for age, sex, tobacco smoking, alcohol drinking, and history of blood transfusion

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Okuno *et al.* 1994 China	Hospital-based case-control study Cases: 186 patients (168 men, 18 women) newly diagnosed with HCC at the Guangxi Medical University Affiliated Hospital, Nanning City, Southern Guangxi, between January 1991 and September 1992 Controls: 48 apparently healthy workers (30 men, 18 women) given a routine physical examination at the same hospital in August 1992	Anti-HCV: 2nd-generation EIA (5.4%/0.0%) HBsAg: RPHA	– (0.6, –)[b]	

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Chang *et al.* 1994 Taiwan	Population-based cohort: 9,775 men aged 30 to 85 from six townships in Taiwan accrued between September 1984 and February 1986. The cohort was actively followed on an annual basis until March 1992 Nested case-control analysis: Cases: 38 cohort members newly diagnosed with primary liver cancer Controls: 152 controls matched by age (within 1 year), township of residence, and date of recruitment	Anti-HCV: 2nd-generation EIA (13.2%/2.6%) HBsAg: RPHA; negative samples rechecked by RIA	88.2 (5.2–1,509) 34.0 (3.5–327.8)	Age-matched OR was further adjusted for HBsAg, vegetable intake, and personal history of chronic liver disease Age-matched OR for HBsAg(-) subjects

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Park *et al.* 1995 Korea	Hospital-based case-control study Cases: 540 HCC patients (433 men, 107 women) admitted to the Kosin University Hospital, in Pusan, Korea, beween July 1992 and February 1994 Controls: 808 apparently healthy residents of Pusan City (431 men, 377 women) who received routine physical examinations at the same hospital between September 1992 and October 1993. All control subjects were free of biochemical or clinical features of liver disease at enrollment.	Anti-HCV: 2nd-generation EIA (14.3%/1.6%) HCV RNA: nested RT-PCR HBsAg: RIA	23.9 (17.4–32.9)	OR adjusted for HBsAg

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Yuan *et al.* 1995 China	Population-based cohort: 18,244 Chinese men, aged 45 to 64, in Shanghai, recruited between January 1986 and September 1989 Nested case-control analysis: Cases: 76 cohort members newly diagnosed with HCC *Controls:* 410 control cohort subjects individually matched to the cases by age (within 1 year), time of blood sample collection (within 1 month), and neighborhood of residence	Anti-HCV: 2nd-generation EIA (1.3%/0.2%) HBsAg: RIA	5.0 (0.3–79.9)	Age-matched OR
Sun *et al.* 1996 Taiwan	Population-based case-control study in seven townships in Taiwan Cases: 58 patients (51 men, 7 women) newly diagnosed with HCC Controls: 225 controls matched by age (within 5 years), gender, and township of residence	Anti-HCV: 2nd-generation EIA (13.8%/4.4%) HCV-RNA: RT-PCR HBsAg: 2nd-generation EIA	8.8 (1.8–43.0) 6.2 (1.4–26.6)	Age- and sex-matched OR was further adjusted for HBsAg Comparable OR for HCV RNA(+)

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Tsai *et al.* 1996 Taiwan	Hospital-based case-control study Cases: 361 patients (303 men, 58 women) newly diagnosed with HCC, who were consecutively admitted to the Kaohsiung Medical College Hospital, in Taiwan, between January 1991 and December 1993 Controls: apparently healthy subjects who entered the same hospital for a physical check-up during the study period; they were individually matched (one control per case) by age (within 5 years) and gender	Anti-HCV: 2nd-generation EIA (29.6%/2.7%) HBsAg, HBeAg, anti-HBe: RIA	59.3 (13.6–258.4)	Age- and sex-matched OR was further adjusted for HBsAg and HBeAg

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Shin *et al.* 1996 Korea	Hospital-based case-control study in Pusan, Korea, August 1990 to August 1993 <u>Cases:</u> 203 patients (159 men, 44 women) newly diagnosed with HCC, admitted consecutively to the Inje University Pusan Paik Hospital <u>Controls:</u> two groups individually matched by age (within 4 years) and sex: 203 apparently healthy subjects who entered the same hospital for a routine checkup, and 203 hospital inpatients free of cancer or liver disease	Anti-HCV: 2nd-generation EIA (11.2%/2.3%) HBsAg, anti-HBc, anti-HBs: RIA	30.3 (6.1–150.6)	Age- and sex-matched OR was further adjusted for HBsAg, *Clonorchis sinensis* in stool, history of blood transfusion, history of acute hepatitis, history of liver fluke, alcohol drinking, and tobacco smoking

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Tanaka *et al.* 1996 Japan	Hospital-based case-control study Cases: 91 patients (73 men, 18 women) newly diagnosed with HCC, aged 40 to 69 and residents of Fukuoka or Saga Prefecture, who were admitted to the Kyushu University Hospital between December 1985 and June 1989 Controls: 410 residents of Fukuoka City (291 men, 119 women), aged 40 to 69, who had a physical examination at a public health center near the Kyushu University Hospital between January 1986 and July 1989. Controls were frequency-matched by age and had no known history of liver disease	Anti-HCV: 2nd-generation IRMA; confirmation by RIBA (78.0%/7.3%) HCV-RNA: RT-PCR HBsAg: RPHA	53.7 (27.1–106.2) 339.6 (96.5–1,195.8)	Age- and sex-adjusted OR Comparable OR for HBsAg(-) subjects only

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Yu *et al.* 1997b China	Multi-region hospital-based case-control study in four areas of southeastern China with a relatively high incidence of HCC Cases: 359 HCC patients [number per location ranged from 71 to 100] Controls: an equal number individually matched by age (within 5 years), sex, and location No information provided on how the cases and controls were identified and selected	Anti-HCV: EIA (Chinese manufactured kits; 1st or 2nd generation not specified) (17.9%/6.5%) HBsAg: EIA (Chinese-manufactured kits)	3.3 (2.0–5.6) 2.4 (0.2–4.8)	Age-, sex-, and location-matched OR Comparable OR for HBsAg(-) subjects only

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Bile *et al.* 1993 Somalia	Hospital-based case-control study in Mogadishu, Somalia, in 1989 Cases: 62 chronic liver disease patients (49 HCC, 13 other liver disease) admitted to two main referral centers Controls: for each case, a hospital control patient, matched for age (within 5 years) and gender, was selected [no further details on control selection was given]	Anti-HCV: 2nd generation EIA (40.3%/6.5%) HBsAg: RIA	9.8 (3.2–30.4) 10.8 (3.0–38.6)	Age- and sex-matched OR Comparable OR in HBsAg(-) subjects

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Cenac *et al.* 1995 Niger	Hospital-based case-control study Cases: 26 Sahelian African HCC patients (19 men, 7 women), who were admitted to the Hospital National, in Niamey, Niger, between June 1983 and June 1985 Controls: 47 Sahelian African patients (24 men, 23 women) admitted to the Department of Internal Medicine in the same hospital during the study period, who had no history of liver disease	Anti-HCV: 2nd-generation EIA (23.1%/6.4%) HBsAg: RIA	4.4 (0.8–21.7)	OR was unadjusted for age or sex

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Kew *et al.* 1997 South Africa	Hospital-based case-control study Cases: 231 black South African HCC patients Controls: hospital controls individually matched (by age within 2 years, sex, race, rural/urban/rural-urban background, hospital, medical versus surgical ward) admitted to four Johannesburg hospitals	Anti-HCV: 2nd- or 3rd- generation EIA (20.8%/5.2%) HCV-RNA: nested RT-PCR HBsAg: RIA	6.1 (2.8–13.7) 6.6 (2.7–15.7)	Age-, sex-, and race-matched OR Comparable OR in HBsAg(-) subjects only
Nomura *et al.* 1996 USA	Population-based male cohort: 5,924 Japanese-American men in Hawaii accrued from 1967 to 1970 and followed for cancer occurrence until 1992 (the Japan-Hawaii Cancer Study) Nested case-control analysis: Cases: 24 cohort members newly diagnosed with HCC Controls: 72 age-matched cohort controls	Anti-HCV: 1st-generation EIA; positive samples retested by RIBA (0.0%/1.4%) HBsAg: RIA	–[c]	

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Yu *et al.* 1997a USA	Population-based case-control study among non-Asians of Los Angeles County, California, aged 18 to 74 Cases: 111 HCC patients Controls: 128 community controls	Anti-HCV: 2nd-generation EIA; confirmation by RIBA (33.3%/3.9%) HBsAg, anti-HBc, anti-HBs: RIA	12.6 (4.7–33.6) 6.5 (2.1–19.9)	Age-, sex-, and race-adjusted OR Comparable OR in HBV(-) subjects
Hadziyannis *et al.* 1995 Greece	Hospital-based case-control study in Athens, Greece Cases: 65 HCC patients Controls: two groups of hospital controls individually matched by age (within 5 years) and gender (65 metastatic liver cancer patients and 65 patients hospitalized for eye, ear, nose, or throat conditions)	Anti-HCV: 2nd-generation EIA; confirmation by RIBA (12.3%/2.3%) HBsAg, anti-HBc, anti-HBs: EIA	7.7 (1.7–35.1)	Age- and sex-matched OR was further adjusted for HBsAg

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Comments
Kaczynski *et al.* 1996 Sweden	Hospital-based case-control study conducted in two hospitals in Goteborg, Sweden from 1984 to 1991 Cases: HCC 73 patients (55 men, 18 women) Controls: 32 patients with other cancers (N = 21) or benign liver disease (N = 11)	Anti-HCV: 3rd-generation EIA; confirmation by RIBA (11.0%/3.0%) HBsAg: RIA	3.7 (0.5–84.5)	OR was not adjusted by age or sex
Donato *et al.* 1997 Italy	Hospital-based case-control study Cases: 172 newly diagnosed HCC patients in the two major hospitals in the province of Brescia, in northern Italy Controls: other hospital patients without a history of liver disease or cancer, frequency-matched by age (5-year age groups), sex, and date and hospital of admission	Anti-HCV: 3rd-generation EIA; confirmation by RIBA (40.1%/6.9%) HCV-RNA: nested RT-PCR HBsAg: EIA	23.2 (11.8–45.7)	OR for concurrent anti-HCV and HCV RNA positivity was adjusted for age, sex, residence, and alcohol intake

[a]No anti-HCV(+) controls were matched to anti-HCV(-) cases; therefore, no finite OR estimate can be calculated.

[b]No controls were anti-HCV(+); therefore, no finite OR estimate can be calculated.

[c]No cases were anti-HCV(+); therefore, no finite OR estimate can be calculated.

Table 3-2. Summary results of the meta-analysis of Donato *et al.* (1998): Odds ratios for hepatocellular carcinoma in subjects testing positive for anti-HCV antibodies versus subjects testing negative

	Number of studies	Anti-HCV positivity OR (95% CI)[a]
Total studies	32 (28 case-control, 3 cohort)	11.5 (9.9–13.3)
HCV seropositivity test:		
1st-generation anti-HCV	11	19.1 (15.2–24.0)
2nd- or 3rd-generation anti-HCV	21	8.2 (6.7–9.9)
Geographical area[b]:		
High risk of HCC (sub-Saharan Africa, East & Southeast Asia)	14	6.2 (4.9–7.8)
Intermediate to low risk of HCC (Japan, Southern Europe, North America)	4	16.8 (11.9–24.1)
Type of controls[b]:		
Hospital	9	6.8 (5.1–9.1)
Community	12	9.0 (7.0–11.6)

[a]Odds ratio (95% confidence interval) for anti-HCV positivity regardless of HBV status.

[b]Only studies using 2nd- or 3rd- generation HCV assays included.

Table 3-3. Results of other case-control studies: Odds ratios for hepatocellular carcinoma subjects testing positive for anti-HCV antibodies versus subjects testing negative

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Remarks
Yuan *et al.* 1996 China	Hospital-based case-control study Cases: 39 HCC patients admitted to the Affiliated Hospital of the Guangxi Medical College in Nanning, Guangxi, China, during 1982 Controls: (1) 41 other patients with age and sex distributions similar to those of the cases and admitted to the same hospital during the study period for reasons other than liver disease and (2) 100 healthy residents of the hospital's catchment area	Anti-HCV: 2nd-generation EIA; confirmation by RIBA (0.0%/0.7%) HBsAg, anti-HBc, anti-HBs: RIA	–[a]	

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Remarks
Okada *et al.* 1998 Japan	Hospital-based case-control study: HCC among patients with chronic liver disease at the National Cancer Center Hospital in Tokyo, Japan, from January 1992 to December 1993 Cases: 141 consecutive HCC patients (110 men, 31 women) with underlying chronic liver disease, aged 25 to 81 Controls: 151 consecutive patients (96 men, 55 women) with chronic liver disease but no evidence of HCC, aged 25 to 79, who were admitted to the same hospital during the study period	Anti-HCV: 2nd-generation EIA (80.0%/71.0%) HBsAg: RPHA Anti-HBs: passive hemagglutination Anti-HBc: EIA	2.3 (1.1–4.9) 4.0 (1.7–9.4)	OR was adjusted for age, sex, HBsAg, and anti-HBc Age- and sex-adjusted OR in HBsAg(-) subjects

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Remarks
Zhang *et al.* 1998 China	Hospital-based case-control study Cases: 152 HCC patients (136 men, 16 women) admitted to four hospitals in Henan Province, China, between January 1994 and October 1995 Controls: 115 patients (99 men, 16 women) admitted to the same hospitals during the study period, with ages similar to those of the cases and judged to be free of liver disease	Anti-HCV: EIA (11.2%/3.5%) Anti-HBc: EIA (unknown generation) HCV RNA: RT-PCR HBsAg, anti-HBs: RIA HBV DNA: PCR	3.5 (1.1–11.6)	OR was unadjusted for age or sex
			2.6 (0.6–12.0)	OR in HBsAg(-) subjects

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Remarks
Yuan *et al.* 1999 USA	Population-based case-control study (Los Angeles Study) Cases: 144 non-Asian HCC patients Controls: 252 community controls of similar age, gender, and race	Anti-HCV: 1st- or 2nd-generation EIA (31.9%/2.0%) HCV RNA: nested RT-PCR HBsAg: RIA Anti-HBsAg, anti-HBcAg: EIA	18.8 (7.1–49.7) – (23.1, –)[b] 4.5 (1.5–13.9)	Age-, sex-, and race-adjusted OR Age-, sex-, and race-adjusted OR in anti-HCV(+)/HCV RNA(+) subjects Age-, sex-, and race-adjusted OR in anti-HCV(+)/HCV RNA(-) subjects

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Remarks
Peters *et al.* 1994 Germany	Hospital-based case-control study Cases: 86 HCC patients (74 men [also given as 71 in one table], 14 women) with an underlying liver cirrhosis, who were seen at the University Hospital of Mainz in Germany from 1986 to 1993 Controls: patients with liver cirrhosis but no evidence of hepatocellular carcinoma, seen at the same hospital during the study period, individually matched by age (within 5 years) and sex	Anti-HCV: 1st- or 2nd-generation EIA (37.0%/22.0%) HBsAg, anti-HBs, anti-HBc, HBeAg, antiHBe, anti-delta antibody: RIA	2.1 (0.8–5.6) 6.4 (1.1–35.2)	Age- and sex-matched OR Comparable OR after adjusting for tobacco and alcohol for HBV(+) subjects only

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Remarks
Goritsas *et al.* 1995 Greece	Hospital-based case-control study Cases: 51 HCC patients (48 men, 3 women) consecutively admitted at the Patras University Hospital in Patras, Greece, between October 1989 and October 1992 Controls: patients seen at the same hospital during the study period, who did not have a history of liver disease or cancer, individually matched by sex and age (within 5 years)	Anti-HCV: 2nd-generatin EIA; confirmation by RIBA (13.7%/2.0%) HBsAg, anti-HBs, anti-HBc: EIA	1.9 (1.1–3.3)	Age- and sex-matched OR was further adjusted for HBsAg, heavy alcohol intake, and tobacco smoking

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Remarks
Tagger *et al.* 1999 Italy	Hospital-based case-control study (Brescia study) Cases: 305 HCC patients Controls: 610 hospital controls	Anti-HCV: 3rd-generation EIA; confirmation by RIBA (42.3%/7.0%) HCV genotype: nested RT-PCR HBsAg, anti-HBs, anti-HBc: EIA	1.5 (0.5–4.5) 26.3 (15.8–44.0)	OR for anti-HCV(+)/HCV RNA(-) after adjusting for age, sex, alcohol intake, and HBV infection Comparable OR for anti-HCV(+)/HCV RNA(+)
Chiesa *et al.* 2000 Italy	Hospital-based case-control study Cases: 142 HCC patients (116 men, 26 women) with liver cirrhosis and 21 HCC patients (19 men, 2 women) without cirrhosis, who were admitted to two main hospitals in Brescia, Italy, between 1995 and 1997 Controls: 610 patients admitted to the same hospitals during the study period, who were free of liver disease	Anti-HCV: 3rd-generation EIA (41.5%/-)[c] (33.3%/-)[c] HBsAg: EIA HBV DNA: PCR HCV RNA: RT-PCR	33.5 (17.7–63.4) 19.7 (6.0–64.8)	Age- and sex-adjusted OR for concurrent anti-HCV and HCV RNA positivity in cirrhosis subjects only Comparable OR in the absence of cirrhosis

Reference and location	Study population	Exposure and anti-HCV prevalence (cases/controls)	OR (95% CI)	Remarks
Kuper *et al.* 2000 Greece	Hospital-based case-control study Cases: 333 HCC patients treated at three teaching hospitals in Athens, Greece, between January 1995 and December 1998 Controls: two groups of hospital controls admitted to the same hospitals during the study period, with age and sex distributions similar to those of the cases: (1) 272 patients with metastatic liver cancer and (2) 360 patients hospitalized for eye, ear, nose, or throat conditions	Anti-HCV: 3rd-generation EIA (15.6%/1.7%) HBsAg: 3rd-generation EIA	23.2 (11.4–47.3) 32.3 (15.0–69.4)	OR was adjusted for age, sex, schooling, and HBsAg Comparable OR for HBsAg(-) subjects only

[a]No cases were anti-HCV(+); therefore, no finite OR estimate can be calculated.

[b]There were no controls in this category; therefore, no finite OR estimate can be calculated.

[c]The number of controls was not given in this study.

Table 3-4. Results of cohort studies: Relative risk of hepatocellular carcinoma in subjects testing postive for anti-HCV antibodies versus subjects testing negative

Reference and location	Cohort/duration of follow-up	Cases/controls or # of cases that developed cancer	Exposure and anti-HCV seroprevalence[a]	Effect	Comments
Kato *et al.* 1994 Japan	Cohort of cirrhosis patients: 401 patients (273 men, 128 women) with cirrhosis, from April 1977 to March 1992, in Nagasaki, Japan, with no history or evidence of HCC at enrollment Follow-up: mean, 4.4 yrs	127 individuals developed HCC	Anti-HCV: 2nd-generation EIA or RIA (53%) HBsAg: RIA	5-year cumulative risk: 36.9% in anti-HCV(+) alone vs. 12.4% in HBsAg(-) /anti-HCV(-)	$P < 0.01$, test for difference in cumulative risk

Reference and location	Cohort/duration of follow-up	Cases/controls or # of cases that developed cancer	Exposure and anti-HCV seroprevalence[a]	Effect	Comments
Tsai *et al.* 1997 China	Cohort of non-alcoholic cirrhosis patients: 400 patients (290 men, 110 women) at the Kaohsiung Medical College Hospital, from January 1989 to December 1994. Follow-up: mean 1,185 person-yrs	80 individuals developed HCC	Anti-HCV: 2nd-generation EIA (31%) HBsAg: RIA	RR[b] = 3.7 95% CI[c] = 1.1–13.1)	RR for anti-HCV(+) alone; adjusted for age, sex, and other potential confounders

Reference and location	Cohort/duration of follow-up	Cases/controls or # of cases that developed cancer	Exposure and anti-HCV seroprevalence[a]	Effect	Comments
Naoumov *et al.* 1997 Multinational	Multinational cohort of 1,438 patients with cirrhosis was assembled from 1978 to 1988 Follow-up: median, 5 yrs, range, 1-6 yrs	Nested case-control analysis: Cases: 72 cohort members (60 men, 12 women) diagnosed with HCC Controls: equal number of cohort controls individually matched by age, sex, country of birth, HBsAg status, duration of follow-up, and etiology of cirrhosis	Anti-HCV: EIA (25%) HCV RNA: RT-PCR HBsAg, anti-HBs, anti-HBc: EIA	RR = 2.1 (95% CI = 1.0–4.4) RR = 2.4 (95% CI = 1.2–5.0)	Multinational cohort of cirrhosis patients; RR matched for age, sex, and country of birth Comparable RR for HCV RNA positivity

Reference and location	Cohort/duration of follow-up	Cases/controls or # of cases that developed cancer	Exposure and anti-HCV seroprevalence[a]	Effect	Comments
Ikeda *et al.* 1998 Japan	Cohort of chronic viral hepatitis patients: 2,215 (1,544 men, 671 women) diagnosed at the Toranomon Hospital, in Tokyo, Japan, from January 1980 to August 1995 Follow-up: median, 4.1 yrs, range, 0.1-16.3 yrs	89 patients developed HCC	Anti-HCV: 2nd-generation EIA (70%) HBsAg: RIA	*P* = 0.01	RR was not given, only *P* value for test of RR = 1
Mori *et al.* 2000 Japan	Population-based cohort: 3,059 residents (981 men, 2,078 women) of Saga Prefecture, Japan, aged 30 or older, during June 1992 Follow-up: median, 4.8 yrs	22 individuals developed cancer	Anti-HCV: 2nd-generation EIA (21%) HBsAg: RPHA	RR = 40.4 (95% CI = 11.7–139.2)	Age- and sex-adjusted RR for high-titer anti-HCV

[a]Percentage prevalence at baseline

[b]Relative risk

[c]95% confidence interval

Table 3-5. Results of studies examining HCV genotype in anti-HCV-positive patients with hepatocellular carcinoma

Reference and location	Study population	Exposure and HCV1b genotype (cases/controls)	OR (95% CI)[a]	Remarks
Yotsuyanagi *et al.* 1995 Japan	Cases: 203 consecutive patients with chronic HCV infection, seen at the University of Tokyo Hospital between 1991 and 1993 Controls: cancer-free chronic HCV patients	HCV genotype: RT-PCR (79.2%/77.1%) Anti-HCV: 2nd-generation EIA HCV RNA: RT-PCR	1.1 (0.5–23)	OR was unadjusted for age or sex
Takada *et al.* 1996 Japan	Cases: three groups of HCV-positive liver-disease patients attending 14 hospitals: (1) 1,922 chronic hepatitis, (2) 356 liver cirrhosis, and (3) 426 HCC Controls: cancer-free chronic HCV patients without cirrhosis	HCV genotype: PCR with type-specific primers or slot-blot hybridization with type-specific probes (80.5%/71.0%)	1.7 (1.3–2.2)	OR was unadjusted for age or sex

Reference and location	Study population	Exposure and HCV1b genotype (cases/controls)	OR (95% CI)[a]	Remarks
Silini *et al.* 1996 Italy	Cases: three groups of anti-HCV-positive liver-disease patients seen at two hospitals in northern Italy between 1991 and 1994: (1) 593 chronic hepatitis, (2) 219 cirrhosis but no evidence of HCC, and (3) 166 cirrhosis and HCC Controls: cancer-free cirrhosis patients	HCV genotype: RT-PCR (72.0%/57.0%) HCV-RNA: RT-PCR Anti-HCV: 2nd-generation EIA; confirmation by RIBA	2.0 (1.3–3.1)	Age- and sex-adjusted OR

Reference and location	Study population	Exposure and HCV1b genotype (cases/controls)	OR (95% CI)[a]	Remarks
Hatzakis *et al.* 1996 Greece	Cases: three groups of anti-HCV-positive liver-disease patients seen at two hospitals in Athens, Greece: (1) 17 HCC, (2) 24 chronic hepatitis and cirrhosis, and (3) 101 chronic hepatitis but no cirrhosis Controls: cancer-free patients with chronic HCV but no cirrhosis	HCV genotype: reverse hybridization (70.6%/26.4%) Anti-HCV: 3rd-generation EIA HCV RNA: RT-PCR	8.3 (1.2–57.5)	Age- and sex-adjusted OR

Reference and location	Study population	Exposure and HCV1b genotype (cases/controls)	OR (95% CI)[a]	Remarks
Lee *et al.* 1996 Korea	Cases: 30 blood donors, 30 patients on maintenance hemodialysis, 33 patients with chronic hepatitis, 15 patients with liver cirrhosis, and 30 patients with hepatocellular carcinoma, all of whom were anti-HCV positive. No information was given on the source of the subjects Controls: cancer-free patients with chronic HCV with or without cirrhosis	HCV genotype RT-PCR: (60.0%/62.5%) HCV RNA: RT-PCR Anti-HCV: 2nd-generation EIA	0.9 (0.3–2.3)	OR was unadjusted for age or sex

Reference and location	Study population	Exposure and HCV1b genotype (cases/controls)	OR (95% CI)[a]	Remarks
Lopez-Labrador *et al.* 1997 Spain	Cases: three groups of patients with HCV-related liver disease seen at a university hospital in Barcelona: (1) 243 asymptomatic chronic hepatitis, (2) 59 decompensated cirrhosis but no evidence of HCC, and (3) 112 liver cirrhosis and HCC Controls: cancer-free patients with asymptomatic chronic HCV	HCV genotype: RFLP (97.3%/72.4%) Anti-HCV: 3rd-generation EIA HCV-RNA: nested RT-PCR	13.8 (4.5–52.9)	OR was unadjusted for age or sex
Bruno *et al.* 1997 Italy	Cohort of HCV(+) cirrhosis patients Cases: 63 consecutive anti-HCV-positive patients with cirrhosis, enrolled January 1989 to December 1990, for the occurrence of HCC	HCV genotype: PCR (86.4%/56.0%) Anti-HCV: 2nd-generation EIA HCV RNA: nested RT-PCR	6.1 (1.8–21.4)	Age- and sex-adjusted RR for genotype 1b after adjusting for alcohol abuse and interferon treatment

Reference and location	Study population	Exposure and HCV1b genotype (cases/controls)	OR (95% CI)[a]	Remarks
Naoumov *et al.* 1997 Multinational	Multinational cohort of cirrhosis patients, nested case-control analysis Cases: 30 cirrhosis patients with HCC Controls: 17 cirrhosis patients positive for HCV RNA	HCV genotype: line probe assay: (50.0%/58.8%) HBsAg, anti-HBs, anti-HBc, anti-HCV: EIA HCV RNA: RT-PCR	0.7 (0.2–2.6)	OR was unadjusted for age or sex

[a]Odds ratio (95% confidence interval) for hepatocellular carcinoma in subjects testing positive for anti-HCV1b antibodies versus subjects testing negative

Table 3-6. Results of individual studies included in the meta-analysis of Donato *et al.* 1998: Odds ratios for hepatocellular carcinoma in subjects stratified jointly by HBV and HCV serology

Study	HBsAg-negative, anti-HCV-negative	HBsAg-positive, anti-HCV-negative		HBsAg-negative, anti-HCV-positive		HBsAg-positive, anti-HCV-positive	
	Cases/controls	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]
Bile *et al.* 1993[c]	14/52	23/6	14.2 (4.9–41.7)	22/3	27.2 (7.1–104)	3/1	11.1 (1.1–116)
Bruix *et al.* 1989[b,d]	20/163	4/3	10.9 (2.3–52.1)	67/10	54.6 (24.3–123)	5/0	–
Cenac *et al.* 1995[c]	5/32	15/12	8.0 (2.4–26.0)	2/2	6.4 (0.7–56.3)	4/1	25.6 (2.4–278)
Chang *et al.* 1994[c]	10/138	23/10	31.7 (11.9–84.7)	4/4	13.8 (3.0–63.6)	1/0	–
Chuang *et al.* 1992[b,d]	16/267	87/104	14.0 (7.8–24.9	13/8	27.1 (9.8–74.8)	12/5	40.1 (12.6–128)
Cordier *et al.* 1993[c]	8/194	138/44	76.1 (34.7–167)	3/2	36.4 (5.3–249)	0/0	–
Coursaget *et al.* 1992[b,d]	25/82	22/51	1.4 (0.7–2.8)	2/0	–	0/1	-
Dazza *et al.* 1993[c,d]	52/163	115/27	13.4 (7.9–22.5)	8/4	6.3 (1.8–21.7)	3/0	-
Di Bisceglie *et al.* 1991[b,d]	80/96	6/0	–	12/2	7.2 (1.6–33.1)	1/0	-
Donato *et al.* 1997[c]	66/292	37/17	9.6 (5.1–18.4)	65/22	13.1 (7.5–22.9)	4/1	17.5 (2.2–439)
Fukuda *et al.* 1993[b]	8/166	17/1	353 (41.6–2,993)	150/9	346 (130–919)	2/0	–
Hadziyannis *et al.* 1995[c]	20/116	37/11	19.5 (8.6–44.5)	5/2	14.5 (2.6–79.9)	3/1	17.4 (1.7–176)

Study	HBsAg-negative, anti-HCV-negative	HBsAg-positive, anti-HCV-negative		HBsAg-negative, anti-HCV-positive		HBsAg-positive, anti-HCV-positive	
	Cases/controls	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]
Kaczynski *et al.* 1996[c]	57/30	0/0	–	7/1	3.7 (0.4–31.3)	0/0	–
Kaklamani *et al.* 1991[b,d]	71/373	42/29	7.6 (4.5–13.0)	29/29	5.3 (3.0–9.3)	43/1	226 (30.6–1,667)
Kew *et al.* 1997[c]	69/197	103/18	23.3 (9.2–59.4)	39/15	6.6 (2.7–15.7)	20/1	82.5 (8.9–762)
Nomura *et al.* 1996[c]	9/69	15/2	57.5 (11.3–294)	0/1	–	0/0	–
Okuno *et al.* 1994[c]	54/43	122/5	19.4 (7.3–51.8)	1/0	–	9/0	–
Park *et al.* 1995[c]	149/753	314/42	37.8 (26.2–54.5)	61/13	23.7 (12.7–44.3)	16/0	–
Pyong *et al.* 1994[b]	9/220	14/9	58.2 (15.3–221)	66/20	92.4 (33.8–252)	1/0	–
Saito *et al.* 1990[b,d]	66/133	49/0	-	136/15	18.3 (9.9–33.6)	2/0	–
Shin *et al.* 1996[c]	40/371	111/14	73.5 (38.6–140)	17/9	17.5 (7.3–41.9)	2/0	–
Simonetti *et al.* 1992 [b,d]	46/197	15/4	16.1 (5.1–50.7)	133/11	51.8 (25.9–104)	18/0	–
Stroffolini *et al.* 1992[c,d]	11/80	11/6	13.3 (4.1–43.3)	38/13	21.3 (8.7–51.8)	5/0	–
Sun *et al.* 1996[c]	8/186	42/29	32.9 (14.5–81.9)	2/10	4.6 (0.6–23.3)	6/0	–
Tanaka *et al.* 1996[c]	3/372	17/8	294 (68.7–1,256)	69/30	340 (96.5–1,196)	2/0	–
Tsai *et al.* 1996[c]	22/278	232/73	40.1 (23.5–69.0)	49/8	77.3 (30.5–204)	58/2	366 (83.4–1,601)
Xu *et al.* 1990 [b,d]	11/46	35/4	36.6 (10.7–125)	1/0	–	3/0	–

Study	HBsAg-negative, anti-HCV-negative	HBsAg-positive, anti-HCV-negative		HBsAg-negative, anti-HCV-positive		HBsAg-positive, anti-HCV-positive	
	Cases/controls	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]
Yang *et al.* 1996[c,d]	15/68	62/11	25.6 (10.9–59.8)	0/1	–	10/0	–
Yu et al. 1991b,d	12/104	101/21	41.7 (19.5–89.2)	5/2	21.7 (3.8–124)	9/0	–
Yu et al. 1997ac	66/123	8/0	–	35/5	13.0 (4.7–44.2)	2/0	–
Yu et al. 1997bc	95/249	184/79	6.1 (4.2–8.9)	19/21	2.4 (1.7–4.8)	42/1	110 (14.9–811)
Yuan et al. 1995c	26/358	49/49	13.8 (7.9–24.1)	0/1	–	1/0	–

[a]Reference group = HBsAg(-)/anti-HCV(-).

[b]Used first-generation anti-HCV tests

[c]Used 2nd- or 3rd-generation anti-HCV or HCV RNA Tests

[d]Reviewed by IARC 1994; not reviewed in the previous sections of this document

Table 3-7. Summary results of the meta-analysis of Donato *et al.* 1998: Odds ratios of hepatocellular carcinoma in subjects stratified jointly by HBV and HCV serology (relative to subjects negative for both HBsAg and anti-HCV antibodies)

	Number of studies	HBsAg-positive anti-HCV-negative OR (95% CI)	HBsAg-negative anti-HCV-positive OR (95% CI)	HBsAg-positive anti-HCV-positive OR (95% CI)
Total studies	32	20.4 (18.0–23.2)	23.6 (20.0–28.1)	135 (79.7–242)
HCV test:				
1st-generation anti-HCV	11	14.7 (11.4–19.1)	34.5 (26.6–45.2)	103 (48.4–251)
2nd-generation anti-HCV	21	22.5 (19.5–26.0)	17.3 (13.9–21.6)	165 (81.2–374)
Geographical area[a]:				
High-risk of HCC (sub-Saharan Africa, East & Southeast Asia)	14	20.8 (17.8–24.3)	11.5 (8.8–15.0)	191 (86.1–494)
Intermediate to low risk of HCC (Japan, Southern Europe, North America)	4	18.8 (11.8–30.3)	31.2 (20.9–47.4)	75.6 (15.6–614)
Type of controls[a]:				
Hospital	9	24.0 (18.4–31.3)	12.6 (9.1–17.4)	34.6 (14.2–101)
Community	12	19.2 (16.2–22.9)	16.2 (12.1–21.7)	420 (143–1,732)

[a]Only studies using 2nd- or 3rd-generation HCV assays included

Table 3-8. Results of other case-control studies: Odds ratios of hepatocellular carcinoma in subjects stratified jointly by HBV and HCV serology (relative to subjects negative for both anti-HBV and anti-HCV antibodies)

	HBV(-)/HCV(-)	HBV(+)/HCV(-)		HBV(-)/HCV(+)		HBV(+)/HCV(+)	
Study	**Cases/controls**	**Cases/controls**	**OR (95% CI)**	**Cases/controls**	**OR (95% CI)**	**Cases/controls**	**OR (95% CI)**
Zhang *et al.* 1998	51/105	84/6	28.8 (11.2–78.8)	5/4	2.6 (0.6–12.0)	12/0	– (5.9, –)[a]
Yuan *et al.* 1999	68/228	30/19	5.2 (2.7–10.0)	24/4	20.1 (6.6–61.2)	22/1	45.5 (5.9–352.2)
Tagger *et al.* 1999	39/221	63/24	21.1 (11.1–40.0)	36/8	35.6 (14.5–87.1)	11/1	132.0 (15.3–890)
Kuper *et al.* 2000	83/574	198/28	53.4 (33.0–86.2)	41/9	32.3 (15.0–69.4)	11/2	46.2 (9.9–216.6)

[a]There were no controls in this category; therefore, no finite OR estimate can be calculated.

Table 3-9. Odds ratios of hepatocellular carcinoma in subjects stratified jointly by HCV serology and alcohol intake level

		Anti-HCV(-) or Anti-HCV(+) and HCV RNA (-)		HCV RNA(+)	
Reference and location	**Alcohol Status**	**Cases/controls**	**OR (95% CI)**	**Cases/controls**	**OR (95% CI)**
Tagger *et al.* 1999 Brescia, Italy	Ethanol intake (g/day)				
	0–40	31/219	1.0	47/18	26.1 (12.6–54)
	41–80	27/157	1.5 (0.7–2.9)	32/7	62.6 (23.3–168)
	>80	120/203	7.3 (4.0–13.1)	42/5	126 (42.8–373)

Table 3-10. Results of case-control studies: Risk of B-cell lymphoma in anti-HCV-positive versus -negative subjects

Reference and location	Study population	Exposure assessment	Prevalence anti-HCV		OR (95% CI)	Remarks
			No. of cases (%)	No. of controls (%)		
Asia						
Izumi *et al.* 1997 Japan	Hospital-based case-control study Cases: 50 patients with B-cell malignancy (25 non-Hodgkin's lymphoma, 4 Waldenstrom's macroglobulinemia, 21 multiple myeloma) Controls: 18 patients with non-B-cell lymphoma No information was given on the source of these patients or their age and sex distributions	Anti-HCV: 2nd-generation EIA HCV RNA: RT-PCR	8 (16.0)	0 (0)	– (0.8, –)[a]	

Reference and location	Study population	Exposure assessment	Prevalence anti-HCV		OR (95% CI)	Remarks
			No. of cases (%)	No. of controls (%)		
Mizorogi *et al.* 2000 Japan	Hospital-based case-control study Cases: 100 patients with B-cell lymphoma, 25 with non-B-cell lymphoma, and 516 with miscellaneous diseases other than liver disease or B-cell lymphoproliferative disorders. All subjects were patients at the Daisan Hospital, in Tokyo, between January 1993 and December 1998	Anti-HCV: 2nd-generation EIA	17 (17.0)	34 (6.6)	2.9 (1.5–5.4)	OR was unadjusted for age or sex

Reference and location	Study population	Exposure assessment	Prevalence anti-HCV		OR (95% CI)	Remarks
			No. of cases (%)	No. of controls (%)		
Americas						
Zuckerman *et al.* 1997 USA	Hospital-based case-control study Cases: 120 consecutive patients with B-cell lymphoma seen at the Los Angeles County-University of Southern California Medical Center between October 1994 and May 1996 Controls: Two groups of controls: (1) 154 patients with hematologic malignancies other than B-cell lymphoma, seen at the same hospital during the study period, and (2) 114 general medicine clinic patients seen at the same hospital during the study period, without hematologic malignancies	Anti-HCV: 2nd-generation EIA HCV RNA: RT-PCR	26 (22.0)	13 (4.9)	5.4 (2.6–11.5)	OR was unadjusted for age or sex

Reference and location	Study population	Exposure assessment	Prevalence anti-HCV		OR (95% CI)	Remarks
			No. of cases (%)	No. of controls (%)		
Collier *et al.* 1999 Canada	Hospital-based case-control study Cases: 100 patients with B-cell lymphoma (10 high grade, 46 intermediate grade, and 44 low grade; 54 men and 46 women) who were seen at the Princess Margaret Hospital, in Toronto, Canada, between February and May 1997 Controls: 100 patients (59 men, 41 women) with gastrointestinal malignancies, of ages similar to those of the cases, who were seen at the same hospital over the study period	Anti-HCV: 3rd-generation EIA HCV RNA: PCR	0 (0.0)[b]	0 (0.0)[b]	–[c]	

Reference and location	Study population	Exposure assessment	Prevalence anti-HCV		OR (95% CI)	Remarks
			No. of cases (%)	No. of controls (%)		
Europe						
Ferri *et al.* 1994 Italy	Hospital-based case-control study Cases: 50 unselected patients (26 men, 24 women) with B-cell lymphoma Controls: 30 Hodgkin's disease patients and 30 healthy subjects of similar ages No information was given regarding the source of subjects.	Anti-HCV: 2nd-generation EIA; confirmation by RIBA HCV-RNA: nested PCR HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe: EIA	15 (30.0)	1 (1.7)	25.3 (3.7–533.0)	OR was unadjusted for age or sex

Reference and location	Study population	Exposure assessment	Prevalence anti-HCV		OR (95% CI)	Remarks
			No. of cases (%)	No. of controls (%)		
Mazzaro *et al.* 1996 Italy	Hospital-based case-control study Cases: 199 consecutive non-Hodgkin's lymphoma patients seen at three medical centers in northern Italy Controls: two groups: (1) 153 patients with other hematological malignancies and (2) 6,917 healthy residents of two towns within the catchment area of the three medical centers. Group 1 controls were similar in age to the cases	Anti-HCV: 2nd-generation EIA; confirmation by RIBA	56 (28.0)	5 (3.1)	11.6 (4.6–31.1)	OR was unadjusted for age or sex

Reference and location	Study population	Exposure assessment	Prevalence anti-HCV		OR (95% CI)	Remarks
			No. of cases (%)	No. of controls (%)		
Vallisa *et al.* 1999 Italy	Hospital-based case-control study Cases: 175 consecutive patients with B-cell lymphoma at the Civil Hospital, in Piacenza, Italy Controls: individually matched (by sex and age within 2 years), obtained from outpatient clinics (N = 175) and hospital wards (N = 175)	Anti-HCV: 2nd-generation EIA; confirmation by RIBA	65 (37.0)	33 (9.4)	5.7 (3.5–9.3)	OR was unadjusted for age or sex

Reference and location	Study population	Exposure assessment	Prevalence anti-HCV		OR (95% CI)	Remarks
			No. of cases (%)	No. of controls (%)		
Pioltelli *et al.* 2000 Italy	Hospital-based case-control study Cases: 300 consecutive patients (145 men, 155 women) with B-cell lymphoma diagnosed at 11 institutions in Lombardy, Italy, between January 1996 and June 1997 Controls: three separate control groups: (1) 600 individually matched patients (by age and sex) without a history of liver disease, cancer, or autoimmune disease, who had never been treated with corticosteroid or immunosuppressive agents, (2) 247 consecutive patients (122 men, 125 women) with solid neoplasm, and (3) 122 consecutive patients (14 men, 108 women) with systemic lupus erythematosus and rheumatoid arthritis, who had been treated with corticosteroid or immunosuppressive drugs for at least 1 year	HBsAg: EIA Anti-HCV: 3rd-generation EIA; confirmation by RIBA	48 (16.0)	51 (8.5)	2.0 (0.7–27.0)	Age- and sex-matched OR

[a]No controls were anti-HCV(+); therefore, no finite OR estimate can be calculated.

[b]Based on 100 cases and 100 controls in the study.

[c]No controls and/or no cases in this category were anti-HCV(+); therefore, no finite OR estimate can be calculated.

Table 3-11. Results of cohort studies: Risk of B-cell lymphoma in anti-HCV-positive versus -negative subjects

Reference and location	Cohort	Exposure assessment	Cohort size/# cases	Sero-prevalence	Duration of follow-up	RR (95% CI)	Remarks
Ohsawa *et al.* 1999 Japan	Cohort of chronic HCV patients: 2,162 (1,328 men, 834 women) with HCV-related chronic liver hepatitis from three hospitals in Osaka, Japan, from 1957 to 1997	Anti-HCV: 3rd-generation EIA HCV RNA: nested PCR	2,162/4	100%	12,405 person-years	2.1 (0.6–5.4)	RR based on observed/expected no. of cases Expected number calculated from sex-, age-, and calendar-year-matched general population rates

[1]Relative risk (95% confidence interval).

4 Studies of Cancer in Experimental Animals

HCV has not been extensively studied in experimental animals because of the lack of suitable models. Furthermore, few closely related viruses can be studied in experimental animals (e.g., GBV-A, -B, and -C). HCV is currently the only member of the *Hepacivirus* genus (see Section 1.2).

Chimpanzees and Chinese tree shrews are the only animals that have been infected with HCV (Gale and Beard 2001, Xie *et al.* 1998). Although laboratory rodents are not susceptible to HCV, some transgenic mouse models can support viral replication, produce viral proteins, and/or develop characteristic liver diseases (Feitelson and Larkin 2001). In addition, Mercer *et al.* (2001) generated mice with chimeric human livers that support HCV replication and release of viral particles. This is a promising model for studying HCV *in vivo* (see Section 1.3.2.3).

IARC (1994) reviewed one case of hepatocellular carcinoma occurring in a chimpanzee that had been inoculated with non-A, non-B hepatitis (discussed below) and concluded that there was inadequate evidence in experimental animals for the carcinogenicity of HCV. No tumors were reported in tree shrews infected with HCV (Xie *et al.* 1998) (see Section 1.3.2.2). This section reviews the available tumor data in chimpanzees and in transgenic mice producing HCV proteins. In addition, injection-site tumors were induced in BALB/c mice challenged with syngenic SP2/0-derived myeloma tumor cells stably producing HCV core protein or NS5 (Encke *et al.* 1998).

4.1 Chimpanzees

Although hepatocellular carcinoma is very rare in chimpanzees, it is important to note that hepatitis C research in these primates did not begin until the mid 1970s and has since been suspended. HCV infection in chimpanzees is milder than typically observed in humans, and only a limited number of animals were ever infected. It is also possible that the follow-up time in these animals was insufficient for hepatocellular carcinoma to develop. Nevertheless, there is one case linking hepatocellular carcinoma with non-A, non-B hepatitis infection (presumed to be HCV) in chimpanzees.

A chimpanzee developed hepatocellular carcinoma, in the absence of other known carcinogenic viruses or environmental factors, seven years after inoculation with serum from a human patient with chronic non-A, non-B hepatitis (Linke *et al.* 1987, Muchmore *et al.* 1988). Subsequent inoculations over the next six years included various plasma-derived products such as the chimpanzee's own acute-phase plasma, factor VIII, coagulation factor concentrate, and anti-thrombin III. This study was the first reported case of hepatocellular carcinoma in chimpanzees. Markers for hepatitis A and B remained negative throughout the study. Liver biopsies collected prior to autopsy strongly suggested non-A, non-B hepatitis. At the time of death, the liver from this chimpanzee was extensively infected with non-A, non-B hepatitis. A homogenate prepared from one of the liver tumors removed at necropsy was injected into another chimpanzee and induced non-A, non-B hepatitis. The causative agent for non-A, non-B

hepatitis was not specifically identified in this chimpanzee, but it was identified as HCV in another chimpanzee (Kohara 2000, Choo *et al.* 1989).

4.2 Mice

Encke *et al.* (1998) used the DNA-based immunization approach to determine whether HCV nonstructural proteins NS3, NS4, and NS5 would induce immune responses in female BALB/c mice. An *in vivo* tumor model was used in one of their experiments to assess cytotoxic T lymphocyte activity. Mice were immunized three times per week with intramuscular (i.m.) injections of either mock DNA or pApNS5 vector. Other groups of mice received three intraperitoneal (i.p.) injections of recombinant NS5 protein or a combination of both (two i.m. injections of pApNS5 and one i.p. injection of recombinant NS5). One week after the last immunization, syngenic SP2/0-derived myeloma tumor cells stably producing NS5 (SP/2NS5-21) were subcutaneously (s.c.) injected into mice in their right flank. Control mice received s.c. injections of SP2/0 cells that produced HCV core protein (SP2/19). Tumor formation at the injection site was assessed at 15 days postinoculation. *In vivo* CTL activity was increased in mice immunized with the NS5-encoding DNA construct, resulting in significantly lower tumor incidence and smaller tumors than in mice immunized with mock DNA, recombinant NS5 protein, or the same syngenic SP2/0 cell line producing a different HCV structural protein. Combined immunization also provided significant, but less effective, protection (Table 4-1).

Table 4-1. Tumor incidence in female BALB/c mice immunized with Mock DNA, pApNS5, or recombinant NS5

Immunization group	Dose (µg) [no. and route]	Tumor challenge	No. with tumors/ no. examined (%)	Tumor size (g ± SEM)
1. Mock DNA	100 [3 i.m.]	SP/2NS5-21	10/10 (100)	1.9 ± 0.2
2. pApNS5	100 [3 i.m.]	SP/2NS5-21	8/20 (40)[a]	0.7 ± 0.1***
3. pApNS5	100 [3 i.m.]	SP/2-19	9/10 (90)	2.2 ± 0.5
4. Recomb NS5	5 [3 i.p.]	SP/2NS5-21	10/10 (100)	1.9 ± 0.2
5. pApNS5 + recomb NS5	100 [2 i.m] + 5 [1 i.p.]	SP/2NS5-21	7/10 (70)[b]	1.1 ± 0.2[c]

Source: Encke *et al.* 1998.

****$P < 0.001$ compared individually with groups 1, 3, and 4 (no comparisons of group 2 with group 5).

[a]Level of significance was not specified.

[b]Authors stated that there was a partial but significant ($P < 0.03$) protection against tumor formation but did not specify whether this applied to tumor incidence, tumor size, or both.

In recent years, transgenic mice that produce the core protein of HCV have developed hepatocellular carcinoma (Moriya *et al.* 1998, Koike *et al.* 2002, Lerat *et al.* 2002); however, this finding has not been observed in all lines of HCV core transgenic mice (Lanford and Bigger 2002). Studies presenting tumor incidence data are briefly discussed below, and the results are summarized in Table 4-2.

Although the precise role of HCV in hepatocarcinogenesis is unclear, evidence exists that the core protein of HCV may be involved by perturbing the regulation of cell proliferation. Moriya *et al.* (1998) and Koike *et al.* (2002) tested this possibility using two independent lines of mice transgenic for the HCV core gene (C21 and C49). Both lines produced the core protein in the liver beginning at birth and continuing for at least 23 months. Levels of core protein in the mouse livers were similar to levels detected in liver samples collected from 42 patients with chronic hepatitis C. Mice from both lineages were sacrificed at 3, 6, 9, 12, and 16 to 19 months in the first study (Moriya *et al.* 1998). This study was subsequently expanded to 23 months (Koike *et al.* 2002). Liver tumors began to appear in mice sacrificed between 16 and 23 months, and the incidence was higher in males than in females (Table 4-2). Mice sacrificed prior to 16 months had steatosis but no signs of inflammatory or neoplastic changes. Tumors began as adenomas, and hepatocellular carcinomas developed later from within the adenomas. The tumor patterns were similar to those observed in humans with chronic hepatitis C infection. The authors concluded that expression of the HCV core gene results in progressive morphological and biochemical changes that lead to hepatocellular carcinoma; therefore, continuous cell death and regeneration are not absolute prerequisites for development of hepatocellular carcinoma. Furthermore, HCV was directly involved in development of liver cancer. They also reported that they did not observe hepatocellular carcinoma in more than 100 transgenic mice in which the HCV envelope gene (coded for by the gene covering the E1 and E2 regions) was introduced over a 24-month period (Koike *et al.* 2002).

Lerat *et al.* (2002) investigated whether production of HCV proteins altered hepatic morphology or function in the absence of inflammation, using transgenic C57BL/6 mice with liver-specific expression of RNA encoding the complete viral polyprotein (FL-N transgene) or viral structural proteins only (S-N transgene). The transgenic mice showed normal growth and development, did not show evidence of hepatic cellular inflammatory infiltrate, and had serum ALT levels not significantly different from those of their nontransgenic littermates. Mice expressing either transgene developed age-related hepatic steatosis, particularly in males. Hepatocellular carcinoma did not occur in nontransgenic littermates or female transgenic mice, but its incidence was significantly elevated in male FL-N/35 transgenic mice (Table 4-2). No tumors occurred in FL-N/35 mice less than 13 months old. Only one male S-N/363 transgenic mouse (> 18 months old) developed cancer; however, only 7 mice in this age group were available for necropsy. In addition, hepatocellular carcinomas also occurred in a few older male transgenic animals from other lineages with active transcription of the complete polyprotein (FL-N/984) or structural proteins only (S-N/866 and S-N/883). However, very few mice from these other lineages were available. The S-N/863 lineage produced higher levels of structural proteins than did the FL-N/35 lineage; however, tumor incidence was higher in the FL-N/35 lineage that also produced nonstructural proteins at low levels. The authors concluded that constitutive expression of viral proteins leads to common pathologic features of hepatitis C in the absence of specific antiviral immune responses. Steatosis was enhanced by production of structural proteins; whereas additional low-level production of nonstructural proteins in the FL-N/35 lineage apparently increased the risk of cancer.

Table 4-2. Tumor incidence in transgenic mice producing HCV proteins

Strain	Age examined (months)	Sex	Tumor incidence[a] (%)	Reference
C57BL/6 controls	16–19	M	0/36 (0)	Moriya *et al.* 1998
	16–19	F	0/33 (0)	
C21 transgenic	16–19	M	7/27 (26)**	
	16–19	F	0/19 (0)	
C49 transgenic	16–19	M	4/13 (31)**	
	16–19	F	2/14 (14)	
C57BL/6 controls	16–23	M	0/56 (0)	Koike *et al.* 2002
	16–23	F	0/NR (0)	
C21 transgenic	16–23	M	11/43 (26)**	
	16–23	F	1/27 (4)	
C49 transgenic	16–23	M	6/19 (32)**	
	16–23	F	3/20 (15)	
C57BL/6 controls	> 13	M	0/24 (0)	Lerat *et al.* 2002
	> 13	F	0/29 (0)	
FL-N/35 transgenic	> 13	M	5/17 (29)**	
	> 13	F	0/20 (0)	
S-N/863 transgenic	> 13	M	1/24 (4)	
	> 13	F	0/18 (0)	

**$P < 0.01$ compared with controls.

[a]NR = not reported.

4.3 Summary

Experimental animal studies of HCV are limited because of the narrow host range. The chimpanzee and tree shrew are the only animals that have been infected with HCV. However, in recent years, the cloned HCV genome has been used to develop transgenic animal models in which to study HCV pathogenesis. Hepatocellular carcinoma has been reported in one chimpanzee infected with HCV for seven years, but not in HCV-infected tree shrews. Hepatocellular carcinoma also developed in a few lines of transgenic mice (primarily males) producing either the HCV core protein or low levels of the complete CV polyprotein.

5 Genotoxicity

No genotoxicity studies of HCV were identified.

6 Other Relevant Data

The mechanism by which HCV causes hepatocellular carcinoma is currently under investigation. This section presents information on the two basic theories of the mechanism of carcinogenic action of HCV: direct versus indirect hepatocarcinogenesis. This section also discusses factors currently being investigated for their role in HCV-related hepatocellular carcinoma, including tumor suppressor genes, oncogenes, and growth factors, and the major hepatocarcinogenic pathways under study for HCV. Finally, cofactors with HCV and their role in the development of hepatocellular carcinoma are discussed.

6.1 Pathogenesis of HCV

The pathogenesis of the liver injury related to hepatitis C is not entirely understood. It is clear that chronic liver injury and inflammation occur with chronic HCV infection and are associated with progressive hepatic fibrosis. This fibrosis may progress to cirrhosis. Once cirrhosis is present, hepatic decompensation may occur, with failure of hepatic synthetic function and portal hypertension.

HCV is notable for a high rate of chronic infection, which occurs in 70% to 80% of those who become infected. Liver biopsies from individuals with chronic HCV infection are notable for the presence of numerous mononuclear cells, at least some of which are CD4+ and CD8+ T lymphocytes. There is little evidence that HCV is directly cytopathic to hepatocytes. Hepatocytes that produce HCV proteins or contain HCV RNA do not appear to be damaged. No more than 20% of hepatocytes appear to be infected with HCV in immune-competent hosts (Krawczynski *et al.* 1992); rather, the liver injury appears to be mediated by the host immune response to HCV. There is no clear correlation between the amount of HCV antigen or RNA within liver cells and the degree of liver injury. Production of HCV core protein in transgenic mice has been noted to result in hepatocellular steatosis, which may be due to mitochondrial injury (Lerat *et al.* 2002). Steatosis, while not the primary form of liver injury, may exacerbate progression of hepatic fibrosis.

HCV-specific cytotoxic T lymphocytes isolated from the liver and peripheral blood mononuclear cells of chronically infected individuals have been reported to recognize multiple epitopes (Wong *et al.* 1998). Results of this study show that the HCV-specific CTL response is quite heterogeneous in people with chronic HCV infection. Even individuals with the same human leukocyte antigen type do not consistently recognize the same epitope. Thus, there does not appear to be an immunodominant response on the CD8+ level in this infection. CD8+ cells do appear to play some role in limiting viral replication. However, these responses are insufficient to eradicate virus completely and may cause liver injury once chronic infection is established. Cytokines produced by both CD4+ and CD8+ cells may play an important role in both inhibiting viral replication and causing liver injury.

HCV-specific CD8+ CTLs have been identified in the circulation of HCV-exposed but seronegative individuals (Koziel *et al.* 1997). This study was based on two subjects who

appeared to have HCV-specific CD4+ and CD8+ responses; these subjects had potential occupational exposures but repeatedly tested negative for serum HCV RNA and failed to produce a humoral response or viremia. Such CTLs are present in much greater proportion within the liver and are thought to mediate hepatocellular injury. In addition, HCV-specific CD4+ helper cells have been identified in chronically infected individuals; they recognize 10- to 25-amino-acid viral peptides in the HLA class II binding groove of antigen-presenting cells (Rehermann 2000). Activated CD4+ T cells in turn stimulate antigen-presenting cells, nonspecific inflammatory cells, antigen-specific CD8+ T cells, and antibody-producing B cells. Most of these interactions are mediated by a variety of cytokines. The humoral immune response is weakly activated in response to HCV infection, and HCV-specific antibodies may play a role in controlling HCV infection but do not seem to be involved in hepatocyte injury.

Liver-cell injury can be identified in biopsy specimens in the form of ballooning degeneration and apoptotic bodies. Specialized studies have shown that the mechanism of liver cell death in chronic hepatitis C is through apoptosis via the Fas pathway (Shackel *et al.* 2002). Hepatic fibrogenesis is mediated by the activation of hepatic stellate cells (HSC), a process that is characterized by a dramatic phenotypic transformation, wherein HSC acquire myofibroblastic features and an enhanced capacity to proliferate (Friedman 1993). The process by which HSC are activated in chronic hepatitis is not known, but it is hypothesized that this activation may occur via cytokine signaling, because of the cytokines produced as part of the immune response to HCV infection. Nonetheless, activated HSC have been detected in liver sections from patients with chronic HCV infection (Khan *et al.* 2001).

Thus, the pathogenesis of HCV-associated liver injury appears to involve many pathways and therefore many genes from multiple pathogenic pathways. Studies using microarray analysis have just begun to explore the variety of gene expression with liver disease. Recent research using cDNA array analysis examined gene expression in HCV-related cirrhosis and found it to be characterized by a proinflammatory, profibrotic, and proapoptotic gene expression profile (Shackel *et al.* 2002).

The pathogenesis of extrahepatic manifestations of HCV infection, such as cryoglobulinemia, membranoproliferative glomerulonephritis, lichen planus, and porphyria cutanea tarda, is uncertain (Sansonno *et al.* 1996). Antibodies to HCV or immune complexes may play a role in all but the last of these complications. Recent studies have demonstrated rearrangement of *bcl*-2 in nearly three-quarters of patients with mixed cryoglobulinemia and in approximately 37% of those with chronic HCV infection without cryoglobulinemia; these results suggest that HCV infection is linked to inhibition of B-cell apoptosis (Zignego *et al.* 2002).

6.2 Direct vs. indirect hepatocarcinogenesis

Although the relationship between HCV infection and hepatocellular carcinoma is well established on epidemiologic grounds, the mechanisms by which chronic HCV infection may lead to hepatocellular carcinoma are not clear. Two general pathways have been suggested. First, it is possible that HCV itself may directly cause cancer. Second, it is

possible that HCV may lead to hepatocellular carcinoma indirectly via hepatic inflammation and regeneration associated with chronic hepatitis.

There is no evidence in humans for a direct pathway of carcinogenesis. The time lag between onset of HCV infection and development of hepatocellular carcinoma is very long, making it difficult to determine a direct relationship between HCV as cause and hepatocellular carcinoma as effect. In one study, investigators described the clinical outcomes of patients with transfusion-associated hepatitis C in which the origin of infection could be dated (Tong *et al.* 1995). They found that hepatocellular carcinoma developed an average of 36 months after the patient's initial diagnosis and approximately 28 years from the onset of HCV infection.

In contrast to HBV and other DNA viruses associated with cancer, in which insertional mutagenesis appears to play a role in carcinogenesis, HCV has an RNA genome that does not integrate into that of infected human cells (Fong *et al.* 1991). Therefore, it is likely that if HCV plays a direct role in hepatocarcinogenesis, it is through the production of viral protein. There is some evidence from *in vitro* and *in vivo* studies suggesting a direct carcinogenic effect for HCV; these studies are described below. However, HCV infection becomes chronic in the majority of cases and leads to immune-mediated liver injury; further, it seems more likely that hepatocellular carcinoma results from the presence of underlying liver inflammation, injury, and fibrosis (Kiyosawa *et al.* 1990).

6.2.1 Possible mechanisms of direct hepatocarcinogenesis

The HCV core protein is a prime suspect in carcinogenesis, as it has been shown to be active in the transcriptional regulation of cellular promoters and proto-oncogenes. For example, it has been demonstrated that the core protein cooperates with *ras* to transform primary rat embryo fibroblasts to a tumorigenic phenotype (Ray *et al.* 1996). The HCV core protein also appears to regulate NF-κB and activating protein-1, as well as the related mitogen-activated protein kinase kinase and c-*jun* N-terminal kinase (Shrivastava *et al.* 1998). Thus, the core protein clearly targets transcription factors known to be involved in the regulation of inflammatory response and cell growth.

6.2.1.1 Apoptosis

The role of HCV core protein with regard to apoptosis is less well defined. One study found that when HCV core protein was produced in HepG2 cells, apoptosis was induced (Kalkeri *et al.* 2001), whereas other investigators have observed that when HCV core protein was produced in Chinese hamster ovary cells, apoptotic cell death was suppressed. Suppression of apoptosis might more clearly lead to malignant transformation than would induction of this mechanism of programmed cell death. In another study, investigators noted that primary human hepatocytes developed an immortalized phenotype when transfected with a vector resulting in production of HCV protein (Ray *et al.* 2000). They subsequently found that inhibition of core expression by antisense oligonucleotides resulted in the prompt death of these hepatocytes through apoptosis (Basu *et al.* 2002).

6.2.1.2 HCV genotypes

Early reports suggested a stronger association of hepatocellular carcinoma with HCV genotype 1b than with other HCV genotypes (Takada *et al.* 1996, De Mitri *et al.* 1995). However, these studies did not correct for the fact that HCV genotype 1b was the prevalent genotype 20 to 30 years ago in Japan, whereas genotypes 2 and 3 have emerged only relatively recently. A recent study found that *neu*, an oncogene, was overexpressed in the livers of patients infected with HCV genotypes 1a and 4, suggesting that these genotypes might play a more prominent role in malignant transformation than others (Zekri *et al.* 2000). More detailed discussion of HCV genotypes is provided in Section 3.

6.2.1.3 Transgenic mice

Experimental animal models, including transgenic mice, have been used to study HCV and hepatocellular carcinoma. Because transgenic mice are immunologically tolerant of HCV core protein and other viral proteins expressed in the liver, results in these mice support the finding that HCV core protein can induce tumors and also plays a major role in hepatocarcinogenesis. For more detailed discussion of experimental animal models, please refer to Section 1.3.2 and Section 4.

6.2.2 Possible mechanisms of indirect hepatocarcinogenesis

In assessing the hypothesis that HCV indirectly results in hepatocellular carcinoma, it is important to recognize that HCV-related hepatocellular carcinoma almost always arises in the presence of cirrhosis. In a retrospective survey of 330 patients with hepatocellular carcinomas surgically resected, there were only 80 in whom the nontumorous liver showed minimal or no hepatic fibrosis (Bralet *et al.* 2000). Interestingly, only two of these patients were found to have HCV infection as a risk factor. Thus, there is a very close connection between cirrhosis and hepatocellular carcinoma (Craig *et al.* 1991). As many as 10% of patients dying with cirrhosis were found to have an unsuspected hepatocellular carcinoma at autopsy. This finding is borne out by transplant studies in which between 3% and 6% of explanted livers have been found to contain small, unsuspected hepatocellular carcinomas (Figueras *et al.* 1997). It is not clear whether all diseases associated with cirrhosis carry an equal risk of hepatocellular carcinoma. The best-documented risk appears to be in cirrhosis with chronic viral hepatitis (HBV and HCV), alcoholism, hemochromatosis, and alpha-1-antitrypsin deficiency. Cirrhotic conditions in which the risk is thought to be lower include Wilson’s disease, primary biliary cirrhosis, and autoimmune hepatitis; however, hepatocellular carcinoma has been well documented in all of these conditions in the presence of cirrhosis, and there may be other explanations for its apparent infrequency (Wang and Czaja 1988, Cheng *et al.* 1992).

The reported rates of hepatocellular carcinoma seem to be higher in case series from Japan than in those from Western Europe (Di Bisceglie 1997). Similar data are lacking from cohorts in the United States. It is not known why hepatocellular carcinoma should develop more frequently in some populations than others. This observation raises the possibility of a role for cocarcinogens, such as alcohol, smoking, or other environmental carcinogens. This idea is supported by the observation that certain cytochrome P450 phenotypes are more closely linked to hepatocellular carcinoma than others among

patients with HCV infection. Chau *et al.* (2000) evaluated the genotypic frequency of the gene *CYP2C19* in 24 Japanese HCV-seropositive, cirrhotic patients who developed hepatocellular carcinoma. The poor metabolizer phenotype was hypothesized to appear in individuals who were homozygous for either of the two mutated alleles (*CYP2C19*2* or *CYP2C19*3)* and in heterozygous patients carrying both the *CYP2C19*2* and *CYP2C19*3* alleles. Using genotyping analysis, 41.7% of the patients (10/24) displayed the poor metabolizer phenotype, including four individuals homozygous for *CYP2C19*2*, two homozygous for *CYP2C19*3*, and four heterozygous for *CYP2C19*2* and *CYP2C19*3*. The frequency of the poor metabolizer phenotype was significantly greater ($P < 0.05$) in these patients than in the corresponding 186 healthy controls.

The question of how cirrhosis results in the predisposition to develop hepatocellular carcinoma has been investigated. The components of HCV-related cirrhosis include inflammation, fibrosis, and hepatocyte regeneration. In one study, serum levels of a variety of pro-inflammatory cytokines were measured in patients with liver disease due to hepatitis C (Kakumu *et al.* 1997). In general, the highest levels of interleukin (IL)-10, IL-15, and soluble TNF-α receptor were found in those patients with hepatocellular carcinoma, suggesting that inflammation has a role in hepatocarcinogenesis. There have been few studies of cellular immune response within the liver of patients with hepatitis C and hepatocellular carcinoma. However, Nakao *et al*. (1997) identified a unique population of CTLs both in the circulation and within the liver that showed hepatic cancer-specific activity. Kawarabayashi *et al*. (2000) noted a decrease in CD56+ T cells and natural killer cells in cirrhotic livers with HCV infection and suggested that this decrease may be what leads to an increased susceptibility to hepatocellular carcinoma.

The prevailing hypothesis of how cirrhosis results in hepatocellular carcinoma is that dysplastic nodules develop within the cirrhotic liver (Takayama *et al.* 1990). These are nodules of hepatocytes greater than 1 cm in diameter (also referred to as macroregenerative nodules, or MRNs, and bounded by fibrosis; they are suspected of being the major premalignant lesion for hepatocellular carcinoma. Areas of cellular atypia develop within these large nodules, leading to dysplasia and, subsequently, foci of well-differentiated hepatocellular carcinoma (Kondo *et al.* 1990).

Among cirrhotic nodules, some stand out as being unusual on gross or microscopic examination. Thus, dysplastic nodules are defined by the International Working Party of the World Congresses of Gastroenterology as a nodular region of hepatocytes at least 1 mm in diameter with dysplasia but without definite criteria of malignancy (International Working Party 1995). Where such nodules are larger than 10 mm, the term "macroregenerative nodule" had been applied. Although this term has been abandoned by the International Working Party, it may still have some value, as clinicians and radiologists often encounter large regenerative nodules that seem to have a propensity to become malignant. Synonyms for this lesion include "large regenerative nodule" and "adenomatous hyperplasia."

Two recent case series have examined the incidence of macroregenerative nodules in liver explants. In one study, 110 sequential explanted cirrhotic livers were examined, and 19 (17.3%) were found to have nodules between 0.8 and 3.5 cm in diameter (Ferrell *et al.*

1992). More than one nodule was present in 10 livers, and a total of 40 nodules were detected. Of these, 12 were hepatocellular carcinomas and 28 were MRNs. In another study, 44 explanted livers were examined, and 48 MRNs larger than 1 cm in diameter were identified in 11 livers (Theise *et al.* 1992). On the cut surface of the liver, these nodules often differed from surrounding cirrhotic nodules in color, texture, or the degree to which they bulged beyond the cut surface of the liver. Both of these studies noted a close association between MRNs and hepatocellular carcinoma within the same liver.

There is some debate as to the origin of dysplastic nodules. It was thought that they were simply “overgrown” cirrhotic nodules. The observation that nearly all dysplastic nodules contain intact portal triads implies that they are not derived from regenerating nodules, but rather might result from growth of a nodule of transformed hepatocytes (Theise *et al.* 1992). A recent study of micronodules from 15 explanted cirrhotic livers from HCV-infected patients made use of laser-capture microdissection to study the human androgen receptor assay (Paradis *et al.* 2000). Based on this assay, approximately half of 112 nodules were shown to be monoclonal in origin. Malignancy develops within large dysplastic nodules at a high rate, ranging from 24% and 45% over a period of several years (Takayama *et al.* 1990, Kondo *et al.* 1990, Borzio *et al.* 1997).

Almost all cases of HCV-related hepatocellular carcinoma are associated with cirrhosis or other severe underlying liver disease, which suggests that it is the longstanding process of inflammation, injury, regeneration, and fibrosis caused by these viral infections that somehow leads to cancer. Certainly the same process could, and probably does, apply to some cases of HBV-related hepatocellular carcinoma, but many more of these cases do not involve cirrhosis. In fact, the younger the patients with HBV-related hepatocellular carcinoma, the less likely they are to have cirrhosis (Kew *et al.* 1982). Hepatocellular carcinoma in children, particularly against a background of HBV infection, often occurs without cirrhosis. However, even if cirrhosis is not present, some evidence of liver injury and regeneration can usually be found. Hepatocellular carcinoma occurring in a totally normal liver is quite unusual, although the fibrolamellar variant of hepatocellular carcinoma arises in the absence of cirrhosis (Craig 1997).

Long-term follow-up studies of patients with cirrhosis due to hepatitis C have found that hepatocellular carcinoma develops at a steady rate, ranging from 1% to 4% per year. One study found that after 20 years, 9.6% to 33.5% of patients with cirrhosis due to hepatitis C will have developed hepatocellular carcinoma (Di Bisceglie 1997). The highest rates have been noted in Japan, whereas in Europe, rates of hepatocellular carcinoma among cirrhotics have ranged between 1.5% and 4% per year (Fattovich *et al.* 1997). The experience most closely resembling that in the United States is observed in Italy. One Italian study on the morbidity and mortality of 384 patients with compensated cirrhosis due to HCV followed patients over a period of 5 to 10 years. In this cohort, the five-year risk of hepatocellular carcinoma was 7% (Fattovich *et al.* 1997).

6.3 Major hepatocarcinogenic pathways

Although the pathways for development of some human cancers have long been well established (such as that for colon cancer), pathways for hepatocellular carcinoma have been more difficult to elucidate. Recently, it has been proposed that human hepatocellular

carcinoma develops by two major pathways (Laurent-Puig *et al.* 2001). The first proposed pathway involves chromosomal instability and is associated with hepatitis B virus infection, and the second pathway is based on a group of more slowly growing tumors that do not show chromosomal instability, but consistently have mutations in the β-catenin gene and loss of heterozygosity in the 8p locus. As discussed below, some evidence exists for a correlation between mutation or altered expression of the β-catenin gene in HCV-related hepatocellular carcinomas.

β-Catenin plays an important role in cell-cell adhesion and in a signaling pathway (the Wnt/wingless pathway) that can be activated by a stabilizing mutation of the β-catenin gene (Hsu *et al.* 2000). Three reports have linked mutations of the β-catenin gene or its expression to HCV-related hepatocellular carcinomas. Huang *et al.* (1999) reported a higher percentage of β-catenin gene mutations in HCV-related hepatocellular carcinomas (9 of 22; 41%) than in hepatocellular carcinomas from unspecified causes (20%). Similarly, Hsu *et al.* (2000) reported a higher frequency of mutations in hepatocellular carcinomas from anti-HCV-positive patients (25%) than in those from HBV-positive patients (9%) or in total hepatocellular carcinomas (13%). Ueta *et al.* (2002) detected mutations in exon 3 of the β-catenin gene in 10 of 57 (17.5%) hepatocellular carcinomas; all 10 patients with gene alterations tested positive for anti-HCV antibodies. In contrast, Laurent-Puig *et al.* (2001) reported that a series of 40 hepatocellular tumors from patients positive for anti-HCV antibodies did not show a significant relationship with any genetic alterations, including β-catenin gene mutations. Given the limited number of tumors examined in the studies cited above, further research in this area will be needed to resolve the apparent difference in findings with respect to an association between β-catenin gene mutation and HCV-related hepatocellular carcinoma.

Many types of chromosomal aberrations have been identified in human hepatocellular carcinomas associated with HCV infection. These may take the form of large-scale chromosomal losses detected by comparative genomic hybridization (Sakakura *et al.* 1999), increased somatic cell mutations (Okada *et al.* 1997), and increased microsatellite instability (Kazachkov *et al.* 1998). The genetic alterations identified in tissue from patients with hepatocellular carcinoma and chromosomal instability include mutations in *axin 1* and loss of heterozygosity in chromosomes 1p, 4q, 16p, and 16q. Other alterations include *p53* mutations and loss of heterozygosity in 13q, 9p, and 6q (Sakakura *et al.* 1999). Again, this pathway is found more frequently in HBV-related hepatocellular carcinoma, but it may play a role in some cases of HCV-related hepatocarcinogenesis.

6.4 Tumor suppressor genes, oncogenes, and growth factors

Tumor suppressor genes such as *RB* and *TP53* may play a significant part in hepatocarcinogenesis. The human tumor suppressor gene *TP53* has been implicated as having a role in several cancers, including colon cancer and hepatocellular carcinoma. Deletion, mutation, or alteration of this gene may result in uncontrolled cellular growth. Mutations have been found within codon 249 of the *TP53* in human hepatocellular carcinoma tissue in as many as 50% of patients in China and southern Africa (Bressac *et al.* 1991, Hsu *et al.* 1991). These mutations appear to occur less frequently in western countries. The reasons for these mutations are not clear, but they may be related to HBV

infection or aflatoxin exposure; they are found much less commonly in HCV-related hepatocellular carcinoma than in HBV-related hepatocellular carcinoma (Hsia *et al.* 1992). Mutation of *TP53* has recently been linked to loss of CD95 gene expression (Volkmann *et al.* 2001). The CD95 (Apo-1/Fas) pathway is involved in apoptosis, suggesting that *TP53* mutations may act in part via inhibition of apoptosis. The process of sequencing *TP53* to determine the presence of mutations is tedious and can perhaps be avoided by immunostaining for tumor protein p53 or by detection of autoantibodies to p53 in serum of patients with hepatocellular carcinoma (Volkmann *et al.* 2001, Stuver *et al.* 2000). Recently, investigators detected anti-p53 antibodies in the sera of 3 of 7 patients with hepatocellular carcinoma and none of 140 patients with chronic HCV infection without hepatocellular carcinoma (Raedle *et al.* 1997).

Several growth factors have been implicated in the development of hepatocellular carcinoma, including transforming growth factors (TGF) alpha and beta, insulin-like growth factors I and II (IGF-I and IGF-II), and hepatocyte growth factor (HGF). The IGF axis has important autocrine, paracrine, and endocrine roles in regulating metabolism and promoting growth. The two IGF ligands, IGF-I and IGF-II, are synthesized by the liver and promote cellular proliferation and inhibition of apoptosis. Measurement of IGF-I levels in sera of patients with hepatocellular carcinoma and in controls showed that levels were significantly lower in patients with hepatocellular carcinoma associated with chronic viral hepatitis than in controls, although the levels did not distinguish clearly between HBV- and HCV-related cancer (Stuver *et al.* 2000). In contrast, other investigators demonstrated what appeared to be increased immunostaining of IGF-II in small hepatocellular carcinomas associated with HCV infection (Sohda *et al.* 1997). Both intrahepatic and serum levels of HGF are elevated in patients with HCV infection. Levels are highest with acute hepatitis C, but also are markedly elevated in cirrhosis and hepatocellular carcinoma. Interestingly, HGF showed little localization to hepatocellular carcinoma cells by immunostaining but was present in infiltrating mesenchymal cells. The mannose 6-phosphate/insulin-like growth factor-II receptor (M6P/IGF2R) plays a role in the activation of the potent growth factor TGF-2 and the degradation of IGF-II. In another study, investigators identified either loss of heterozygosity at the *M6P/IGF2R* locus (which maps to chromosome 6q26-q27) or point mutations in the remaining allele in a large proportion of human hepatocellular carcinomas (De Souza *et al.* 1995). These data suggest that *M6P/IGF2R* acts as a tumor-suppressor gene in human liver carcinogenesis.

A variety of other tumor-related genes have been found to be mutated or to have increased expression in hepatocellular carcinoma. Among these are the *bcl*-2 family, the novel malignancy-associated gene, the tumor suppressor gene *RB*, and a Myc-binding protein (MBP-1) gene (Frommel *et al.* 1999, Ljubimova *et al.* 1998, Hsia *et al.* 1994, Fan *et al.* 2001). In most cases, studies of these tumor-related genes have not clearly separated HCV-related from other hepatocellular carcinomas. A recent genome-wide analysis of gene expression in human hepatocellular carcinoma was performed using cDNA microarrays. It is apparent that there is substantial differential gene expression between HBV- and HCV-related hepatocellular carcinoma (Okabe *et al.* 2001).

6.5 Cofactors with HCV

Although it is clear that chronic HCV infection is sufficient to cause hepatocellular carcinoma in a cirrhotic patient, there is substantial evidence that other cofactors may play a role in augmenting the development of cancer. The most frequently cited cofactor is coinfection with HBV (Kew *et al.* 1997, Sugawara *et al.* 1999) (see Section 3.3.5). As summarized above, chronic coinfection with HBV and HCV has a synergistic effect on the risk of hepatocellular carcinoma (i.e., the risk is greater than the sum of the separate risks associated each infection) (Donato *et al.* 1998; Tables 3-6 and 3-7). It was calculated that coinfection with HBV increased the relative risk of hepatocellular carcinoma by more than 10-fold in patients with chronic HCV (Kew *et al.* 1997). The mechanisms by which this synergism might occur are not known. Evidence is accumulating that HBV may interfere with HCV replication, and vice versa. Virus titers are lower in patients coinfected with HBV and HCV than in patients infected with either virus alone (Jardi *et al.* 2001, Kazemi-Shirazi *et al.* 2000, Mathurin *et al.* 2000); however, the mechanism by which this occurs has not been defined. Futhermore, it is unknown how this viral interference might explain the poorer prognosis of coinfected patients. Clearly, coexistent HBV infection may exacerbate the liver disease activity from hepatitis C, resulting in an earlier onset of cirrhosis and increasing the risk of hepatocellular carcinoma. In studies in the 1980s and 1990s, HBV DNA was observed within hepatocellular carcinomas and the adjacent tissue of many patients with hepatocellular carcinoma who were seronegative for HBsAg. However, a more recent study reported the presence of HBV in only 4% of the HBsAg-seronegative patients tested. The authors suggested that these results could be explained by either false-positive results in the earlier studies due to bacterial contamination in the Southern blot method or false classification of the patients as being HBsAg-negative in the earlier studies (Sugawara *et al.* 1999). The X transcript of HBV has been detected within the liver tissue of Japanese patients with HCV-related hepatocellular carcinoma, suggesting that the X gene might promote hepatocarcinogenesis (Tamori *et al.* 1999).

Another viral coinfection with HCV that has been implicated in causing hepatocellular carcinoma is Epstein-Barr virus infection. EBV DNA was detected at significant levels in tumor tissue from approximately one-third of patients with hepatocellular carcinoma in a Japanese study (Sugawara *et al.* 2000). However, an association between hepatocellular carcinoma and EBV infection could not be confirmed in a study conducted in the United States (Chu *et al.* 2001).

Hormonal influences may affect the risk of hepatocellular carcinoma in patients with chronic hepatitis C. Men are several times more likely to develop hepatocellular carcinoma than women, particularly premenopausal women (Bosch *et al.* 1999). A prospective study of male cirrhotics in Japan suggests that those with higher testosterone and lower estrogen levels in serum are at greatest risk of hepatocellular carcinoma (Tanaka *et al.* 2000). Diabetes mellitus has been reported to increase the risk of hepatocellular carcinoma among patients with HCV infection or other risk factors (i.e., HBV infection or alcoholic cirrhosis) (El-Serag *et al.* 2001). However, as described in Section 3.3.1.4, adjustment for history of diabetes did not materially change the HCV-hepatocellular carcinoma association in the study by Hadziyannis *et al.* (1995).

Other factors such as alcohol, smoking, and betel-quid chewing (chewing of a mixture of the nut of the *Areca catechu* palm, the leaf or inflorescence of *Piper betle*, and a slaked-lime paste) have all been implicated in increasing the risk of hepatocellular carcinoma in the presence of chronic viral hepatitis (Chiesa *et al.* 2000, Kuper *et al.* 2000, Tsai *et al.* 2001). Among patients with HCV-related cirrhosis, it has been observed that liver iron deposition is more frequent and more prominent in those with hepatocellular carcinoma than in those who do not have hepatocellular carcinoma (Chapoutot *et al.* 2000). The mechanism by which iron might contribute to HCV-related hepatocellular carcinoma is not known, but it might act by increasing oxidative stress, a factor independently associated with hepatocellular carcinoma (Sumida *et al.* 2000, Schwarz *et al.* 2001).

6.6 Summary

Most individuals infected with HCV develop chronic hepatitis, which is associated with chronic liver injury and inflammation. Liver injury appears to be a result of the host immune reaction to the virus rather than damage from the virus itself. Chronic infection usually results in progressive hepatic fibrosis, which may progress to cirrhosis and other disease states. Many pathways and many genes appear to be involved in the pathogenesis of HCV-associated liver injury.

The mechanisms of HCV-related hepatocarcinogenesis have not been elucidated. HCV may directly cause cancer or may cause cancer indirectly as a result of hepatic inflammation and regeneration associated with chronic hepatitis. Because HCV is an RNA virus, and thus does not integrate into the host DNA, direct mechanisms would most likely involve production of viral protein. The HCV core protein is the current leading suspect, based on its role in regulating cellular promoters and proto-oncogenes and on studies in transgenic mice. *In vitro* studies have reported that the core protein cooperates with ras to transform primary rat embryo fibroblasts to a tumorigenic phenotype. The HCV core protein also induces tumors in some but not all lines of transgenic mice. The roles of other viral proteins in hepatocarcinogenesis remain largely unexplored.

HCV-related liver cancer almost always arises in the presence of cirrhosis, suggesting the importance of indirect mechanisms such as inflammation, fibrosis, and hepatocyte regeneration in cancer development. The prevailing hypothesis of how cirrhosis results in hepatocellular carcinoma is that dysplastic nodules develop within the cirrhotic liver.

The pathway of hepatocarcinogenesis related to HCV infection appears to be different from that associated with HBV infection. Whereas HBV-associated liver cancer is characterized by genetic instability, several but not all studies have reported an association between mutations in the β-catenin gene and HCV-associated liver cancer; however, these studies were based on small numbers of tumors. The interplay between these mutations and underlying liver disease (i.e., presence, duration, and severity of underlying liver disease) remains to be examined.

Epidemiologic studies have shown that there is a synergistic risk of hepatocellular carcinoma in individuals chronically infected with both HBV and HCV, but the mechanism of this interaction is unknown. Coinfection with HBV may exacerbate the

liver disease activity from hepatitis C, and thus increase hepatocellular carcinoma risk. Nonviral cofactors that may increase the risk of liver cancer in HCV patients include alcohol, smoking, and betel-quid chewing.

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Glossary of Hepatitis- or Virology-related Terms

Acute hepatitis: Newly acquired symptomatic hepatitis virus infection, usually less than 6 months in duration.

Antigen: Any substance that, as a result of coming in contact with appropriate cells, induces a state of sensitivity and/or immune responsiveness after a latent period (days to weeks) and which reacts in a demonstrable way with antibodies and/or immune cells of the sensitized subject *in vivo* or *in vitro*.

Carrier: A person or animal that harbors a specific infectious agent without visible symptoms of the disease. A carrier acts as a potential source of infection.

Chronic hepatitis: Any of several types of hepatitis persisting for ≥ 6 months, often progressing to cirrhosis; this condition is characterized by abnormal levels of liver enzymes and inflammatory changes on liver biopsy.

Clearance: Removal of a substance, such as viral particles, from the blood (e.g., by renal excretion).

DNA virus: A set of viruses that use DNA for the storage of their genetic information.

Flaviviridae: A family of enveloped single-stranded positive sense RNA viruses formerly classified as the "group B" arboviruses.

Hepacivirus: Genus for HCV of which HCV is the only member; within the genus, numerous isolates of HCV are currently grouped into 6 major genotypes and several subtypes.

Hepadnavirus: A group of animal DNA viruses including viruses of ducks, woodchucks, squirrels, and others as well as the virus causing hepatitis B in humans.

Hepatitis C core protein: The putative nucleocapsid of the HCV polyprotein. This 191 amino acid region is highly conserved and comprises several T-cell and B-cell epitopes.

Infectivity: The characteristic of a disease agent that embodies capability of entering, surviving in, and multiplying in a susceptible host.

Non-A, non-B hepatitis: Previous designation for HCV.

Nucleocapsid: A unit of viral structure, consisting of a capsid (protein coat) with the enclosed nucleic acid.

Pestivirus: A genus of viruses composed of the classical swine fever virus, bovine viral diarrhea virus, and related viruses; these viruses are animal pathogens and are especially important in livestock.

Productive infection: The result of a virus' ability to yield an infection.

RNA virus: A specialized set of viruses that use RNA, rather than DNA, for the storage of their genetic information. HCV is an RNA virus.

Seroconversion: Development of antibodies in the blood of an individual who previously did not have detectable antibodies.

Viral envelope: The outer structure, composed of two layers of lipids, that encloses the nucleocapsids of some viruses; the envelope may contain host material.

Viral titer: The concentration of infectious viral particles per milliliter of suspension fluid.

Viremia: The presence of a virus in the bloodstream.

Virion: The complete viral particle, found extracellularly and capable of surviving and infecting a living cell.