HEPATITIS C VIRUS

1. Exposure Data

1.1 Structure and biology of hepatitis C virus (HCV)

1.1.1 *Structure of the virus*

The etiological agent of most cases of post-transfusion hepatitis and a variable proportion of sporadic non-A, non-B hepatitis was discovered in 1989, by recombinant cDNA immunoscreening of serum from a chimpanzee chronically infected with a contaminated human factor VIII concentrate (Choo *et al.*, 1989). The agent was termed hepatitis C virus (HCV). It is a positive-strand RNA virus distantly related to the pestiviruses and flaviviruses on the basis of similar biophysical characteristics, genome organization, hydrophobicity plots and consensus sequences (Miller & Purcell, 1990; Han *et al.*, 1991; Koonin, 1991).

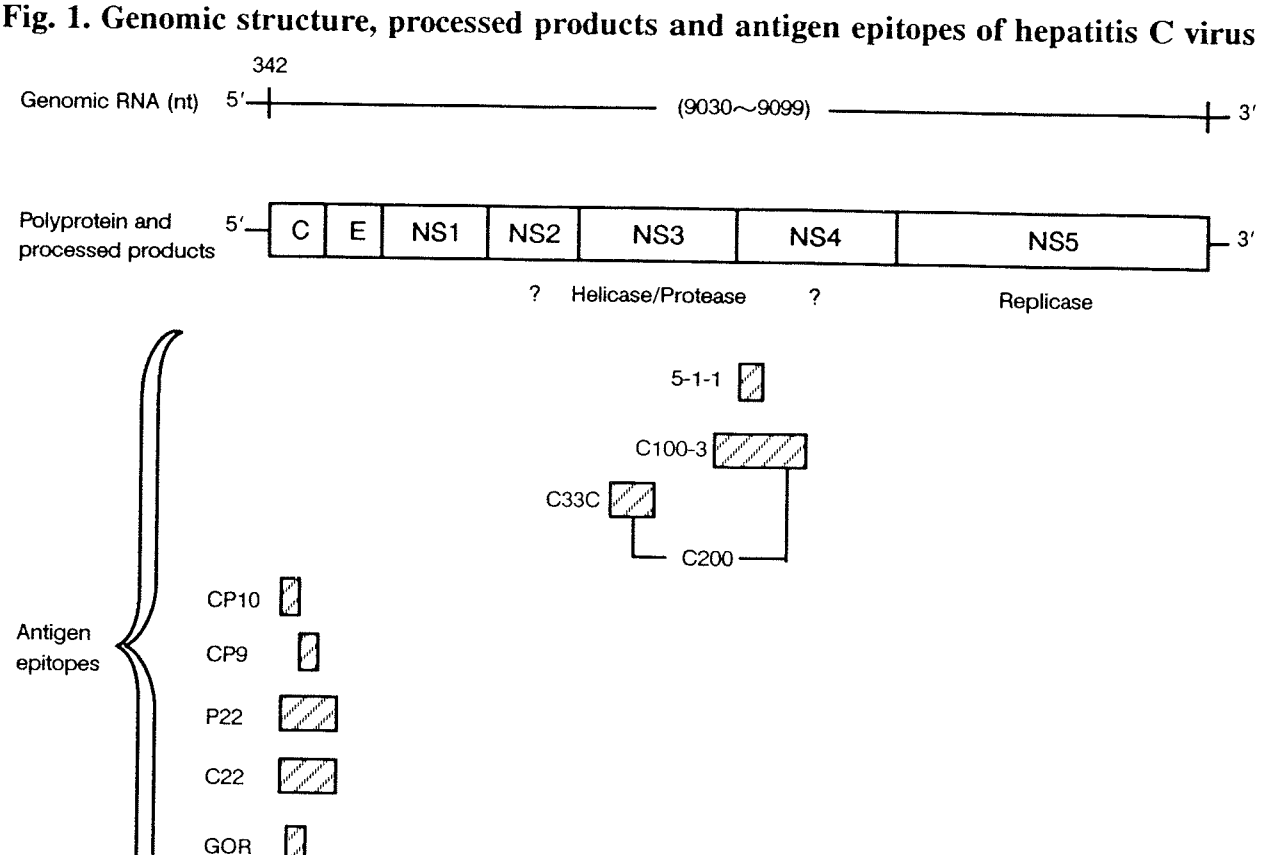
1.1.2 *Structure of HCV genome and gene products*

The genomic organization and characterization of HCV have been described (Choo *et al.*, 1991; Han *et al.*, 1991; Cha *et al.*, 1992). The viral genome is a positive-strand RNA molecule about 9.4 kilobases long (Fig. 1), which is translated into a viral polyprotein. The prototype HCV nucleotide sequence is called HCV-1 (Choo *et al.*, 1991). The single large open reading frame encodes a polyprotein precursor of about 3010 amino acids, which contains co-linearly structural and nonstructural proteins. Putative boundaries are assigned that separate the 5′ untranslated region, the core protein, the glycoprotein envelope 1 (E1), the nonstructural protein 1/envelope 2 (NS1/E2), the nonstructural proteins 2–5 (NS2, NS3, NS4 and NS5) and the 3′ untranslated region.

1.1.3 *Replication and gene expression of HCV*

HCV RNA is transcribed into minus-strand RNA, the putative replication intermediate. HCV does not appear to produce DNA replicative intermediates, and integrated viral sequences have not been found in the host genome (Choo *et al.*, 1989). Viral proteins or viral particles have not been identified in serum from HCV-infected individuals; however, viral antigens have recently been detected in infected hepatocytes by immunohistochemical analysis (Hiramatsu *et al.*, 1992; Krawczynski *et al.*, 1992). Apart from a study using a human T-cell line (Shimizu, Y.K. *et al.*, 1992), infection of cells with HCV *in vitro* has not been reported.

Fig. 1. Genomic structure, processed products and antigen epitopes of hepatitis C virus



nt, nucleotide; C, core protein; E, envelope; NS, nonstructural protein
From Greenwood & Whittle (1981); Alter, H.J. *et al.* (1989); Kuo *et al.* (1989); McFarlane *et al.* (1990); Mishiro *et al.* (1990); Okamoto *et al.* (1990a); Chiba *et al.* (1991); Houghton *et al.* (1991); Suzuki *et al.* (1991); Watanabe *et al.* (1991a,b); Weiner *et al.* (1991a); Bresters *et al.* (1992); Claeys *et al.* (1992); Kotwal *et al.* (1992a); Matsuura *et al.* (1992); van der Poel *et al.* (1992); Watanabe *et al.* (1993)

It has been possible to identify viral proteins by transcription of RNA from cloned HCV cDNA as well as by transfection of cell lines (Harada *et al.*, 1991; Kumar *et al.*, 1992) *in vitro*, followed by translation of the RNAs *in vitro*. HCV core and envelope proteins, encoded by the NS1/E2 region of the viral genome, have been expressed *in vitro* in *Escherichia coli* (Mita *et al.*, 1992), in insect cells (Matsuura *et al.*, 1992) and in mammalian cells (Matsuura *et al.*, 1992; Spaete *et al.*, 1992). The E2 protein appears to have an amino-terminal hypervariable region that may be the target of immune selection of HCV variants and may be found sequentially in infected individuals (Weiner *et al.*, 1992). The full-length protein NS1/E2 appears to be cell-associated and is not secreted; in contrast, C-terminal truncated proteins were detected extracellularly and may be relevant targets for the host immune response and therefore potential subunit vaccine candidates (Spaete *et al.*, 1992). In the natural course of HCV infection, however, antibodies to the NS1/E2 protein are detected infrequently and do not serve as evidence of viral clearance (Matsuura *et al.*, 1992; Mita *et al.*, 1992).

1.1.4 *HCV animal models*

HCV has been detected in humans and has been successfully transmitted to chimpanzees. At present, the chimpanzee is the only established animal model for non-A, non-B hepatitis (Alter *et al.*, 1978; Tabor *et al.*, 1978; Bradley *et al.*, 1979; Wyke *et al.*, 1979; Yoshizawa *et al.*, 1980) and HCV infection specifically (Shimizu *et al.*, 1990). The natural course of HCV infection has been studied in this animal model (Bradley *et al.* 1990; Abe *et al.*, 1992; Beach *et al.*, 1992; Farci *et al.*, 1992a; Hilfenhaus *et al.*, 1992; Shindo *et al.*, 1992a). Most importantly, studies in champanzees reveal a lack of protective immunity against reinfection with HCV (Farci *et al.*, 1992b; Prince *et al.*, 1992). In contrast to hepatitis B virus (HBV), no naturally occurring HCV-related animal virus has been identified.

1.1.5 *Genotypes of HCV*

After the initial discovery of HCV (Choo *et al.*, 1989), viral isolates from different parts of the world were sequenced, and a huge amount of information on HCV diversity has been published. Complete HCV cDNA sequences have been established for isolates from the USA, including the initial clone HCV-1 (Choo *et al.*, 1991) and the HCV-H virus (Inchauspé *et al.*, 1991), and for isolates from Japan, including HCV-J (Kato *et al.*, 1990), HCV-BK (Takamizawa *et al.*, 1991), HCV-J4 (Okamoto *et al.*, 1990a, 1992a), HCV-J6 (Okamoto *et al.*, 1991) and HCV-J8 (Okamoto, 1992; Okamoto *et al.*, 1992a). Partial HCV cDNA sequences are known for isolates from the USA (Weiner *et al.*, 1991), Japan (Enomoto *et al.*, 1990; Maéno *et al.*, 1990; Okamoto *et al.*, 1990a; Takeuchi *et al.*, 1990a; Hijikata *et al.*, 1991; Tsukiyama-Kohara *et al.*, 1991), Thailand (Mori *et al.*, 1992), China (Chen *et al.*, 1992; Liu *et al.*, 1992; Wang Y. *et al.*, 1992), France (Kremsdorf *et al.* 1991; Li *et al.*, 1991), Germany (Fuchs *et al.*, 1991) and Scotland (Chan, S.-W. *et al.*, 1992).

Comparison of the sequence of the original HCV-1 isolate from the USA (Choo *et al.*, 1989) with a Japanese isolate, HCV-J (Kato *et al.*, 1990), revealed that these HCVs differ both in nucleotide and polypeptide sequence (Kubo *et al.*, 1989; Takeuchi *et al.*, 1990b; Choo *et al.*, 1991). On the basis of nucleotide sequence homology, several genotypes have been identified throughout the world (Enomoto *et al.*, 1990; Houghton *et al.*, 1991; Cha *et al.*, 1992; Chan, S.-W. *et al.*, 1992; Okamoto *et al.*, 1992a,b). On the basis of nucleotide sequence homology of whole sequenced HCV isolates, they were classified into type I (1a), type II (1b), type III (2a) and type IV (2b). Provisionally, type V (3a) and type VI (3b) isolates were reported on the basis of data on partially sequenced genomes.

Apart from the geographic distribution of HCV genotypes mentioned above, recent evidence suggests that HCV exists in infected individuals as different but related genomes, known as quasispecies (Martell *et al.*, 1992; Murakawa *et al.*, 1992; Tanaka, T. *et al.*, 1992; Weiner *et al.*, 1992). Researchers have proposed many classification schemes based primarily on nucleotide sequence homology using different regions of the genome. There is no universally agreed classification.

1.1.6 *Host range and target cells of HCV infection*

The host range of HCV is very narrow, as HCV infects only humans and chimpanzees. The molecular basis of this narrow host range is not known.

In permissive hosts, viral antigens and nucleic acids are found primarily in serum and liver cells. In infected liver tissues, HCV antigens have been detected by immunohistochemical analysis (Hiramatsu *et al.*, 1992; Krawczynski *et al.*, 1992), and RNA has been found in liver and serum by molecular techniques (Fong *et al.*, 1991; Akyol *et al.*, 1992; Bresters *et al.*, 1992; Diamantis *et al.*, 1992; Hosoda *et al.*, 1992; Lamas *et al.*, 1992; Negro *et al.*, 1992; Takehara *et al.*, 1992). HCV RNA has also been detected in peripheral blood mononuclear cells (Qian *et al.*, 1992; Hsieh *et al.*, 1992; Wang, J.-T. *et al.*, 1992; Zignego *et al.*, 1992). Experimental evidence was obtained recently for in-vitro infection and replication of HCV in a human T-cell line (Shimizu, Y.K. *et al.*, 1992).

The biological significance of HCV in cells other than hepatocytes remains largely undefined, however. Blood mononuclear cells may play a critical role in reactivation episodes during chronic HCV infection, after interferon treatment of chronic hepatitis C (Qian *et al.*, 1992) and in reinfection after liver transplantation (Read *et al.*, 1991; Ferrell *et al.*, 1992a; Belli *et al.*, 1993).

1.2 Methods of detection

The detection of infection is based upon assays for viral antibodies and viral nucleic acids. In contrast to HBV infection, no assay system is yet available commercially for detection of HCV antigens in serum or plasma, although they can be detected in serum by research techniques, such as enzyme-linked immunosorbent assay (ELISA) (Takahashi *et al.*, 1992).

1.2.1 *In serum and plasma*

Tests for anti-HCV first became available in 1989. These are known as first-generation assays and had limited sensitivity and specificity; they have been superseded by improved second-generation assays. Neither test distinguishes between current and past HCV infection.

(*a*) *The first-generation anti-HCV assay*

In this assay, C100-3 antigen, a recombinant antigen derived from the NS3–NS4 region of the HCV genome (Fig. 1), is used to capture anti-HCV. The labelled anti-HCV is then detected by a second labelled antibody to human immunoglobulin (Kuo *et al.*, 1989). This assay is now available commercially in the ELISA format. In the USA, anti-C100-3 was used to detect anti-HCV in radioimmunoassays in about 90% of blood units implicated in post-transfusion hepatitis (Alter, H.J. *et al.*, 1989).

Screening for anti-C100-3 before blood transfusion reduced the number of cases of post-transfusion non-A, non-B hepatitis in Japan by 60–80% (Japanese Red Cross Non-A, Non-B Hepatitis Research Group, 1991). This test also detected anti-HCV in about 60% of HCV RNA-positive blood donors (Watanabe *et al.*, 1993).

The specificity of the assay is reduced by freezing and thawing serum or plasma and by the presence of high immunoglobulin levels (McFarlane *et al.*, 1990). The latter aspect is particularly important, since in many etiological forms of chronic liver disease immunoglobulin levels are typically elevated, and in individuals living in tropical areas immuno-

globulin levels can be very high owing to chronic parasitic infections (Greenwood & Whittle, 1981).

(*b*) *The second-generation anti-HCV assays*

In second-generation anti-HCV assays, a recombinant antigen of the non-structural NS3 region, named C33c, and a recombinant antigen of the nucleocapsid (core) region, named C22, were added to the previously used C100-3 antigen for a second-generation ELISA. In another assay, C200 antigen expressed as one polypeptide comprising C100-3 and C33c is used as antigen together with C22 (Bresters *et al.*, 1992; van der Poel *et al.*, 1992). Agglutination tests have also been developed in which gelatin particles coated with second-generation antigens (particle agglutination) and fixed erythrocytes coated with second-generation antigen (passive haemagglutination) have been used to measure anti-HCV. More than 98% of RNA-positive serum samples are detected in second-generation assays for anti-HCV as compared with about 60% in first-generation assays (Watanabe *et al.*, 1993).

Several other systems using other antigens, e.g. NS5, have been described. The second-generation assays are more specific and sensitive than the first-generation assays and to a large degree overcome the limitations mentioned above.

(*c*) *Confirmatory tests*

Confirmatory recombinant immunoblot assays (RIBA) and neutralization assays were developed using different viral antigens. The inclusion of additional antigens and a format different from the ELISA improves the specificity of the test. Confirmatory tests give positive results in more than 90% of patients with chronic liver disease or post-transfusion hepatitis tested by ELISA (Suzuki *et al.*, 1991; Watanabe *et al.*, 1991a).

(*d*) *Other anti-HCV assays*

Assays have also been based on core and core-related antigens (see Fig. 1), including CP9 (Okamoto *et al.*, 1990b), CP10 (Okamoto *et al.*, 1992c), P22 (Chiba *et al.*, 1991), HCV core (Claeys *et al.*, 1992), HCV-SP (synthetic polypeptide) (Kotwal *et al.*, 1992a), N14 (Watanabe *et al.*, 1993) and GOR (Mishiro *et al.*, 1990; Watanabe *et al.*, 1991b). The relevance of these assays to the natural history of the disease remains to be established.

(*e*) *HCV RNA*

HCV infection can also be assessed by detecting HCV RNA by reverse transcription (RT) and the polymerase chain reaction (PCR), which is highly sensitive and has been used for early diagnosis: Quantitative PCR can be used to detect 5–30 molecules of synthetic HCV RNA (Hagiwara *et al.*, 1993). The detection limit of PCR is at present about 10 chimpanzee infectious doses per millilitre of serum (Okamoto *et al.*, 1990c).

Well-controlled procedures for handling samples, extraction and purification of nucleic acids, avoidance of laboratory contamination and use of appropriate negative and positive controls are essential prerequisites for the PCR assay. Selection of primers from the highly conserved 5′ non-coding region is also important for sensitivity and has allowed identification of a broad range of genotypes (Okamoto *et al.*, 1990c).

Testing by PCR has become the 'gold standard' for some workers. The results of these tests correlate well with the risk for transmitting post-transfusion hepatitis, with those of

second-generation anti-HCV assays and with liver histology and are useful in monitoring the response of patients to interferon therapy. The test suffers from the risk for contamination, however, and reproducibility between laboratories has been poor (Zaaijer *et al.*, 1993).

1.2.2 *In liver tissues*

(*a*) *HCV antigen*

HCV antigen can be detected immunohistochemically using fluorescein isothiocyanate-labelled immunoglobulin G fractions from chimpanzee and human sera that are strongly reactive with recombinant structural and non-structural proteins of HCV. In one study, the antigen was localized in the cytoplasm of hepatocytes in all nine chimpanzees with acute hepatitis C, in 5/10 chimpanzees with chronic HCV infection and in 11/12 patients with chronic hepatitis C. Direct immunomorphological evidence for the presence of HCV antigen deposits in hepatocytes using fluorescein isothiocyanate-labelled polyclonal anti-HCV antigen probe was established in absorption experiments using recombinant HCV non-structural proteins. The putative HCV NS3 protein was the most readily detected component of HCV in liver cells (Krawczynski *et al.*, 1992).

(*b*) *HCV RNA*

HCV RNA can be detected in liver biopsy samples from patients with chronic hepatitis C by RT–PCR and confirmed by Southern blotting. Shieh *et al.* (1991) used primers from both NS3 and core regions and detected the NS3 region more frequently than the core region. HCV RNA was localized by in-situ hybridization in the cytoplasm of hepatocytes in liver biopsy samples obtained from patients with chronic non-A, non-B hepatitis who were sero-positive for anti-HCV.

The presence of minus-strand HCV RNA was tested in blood and liver specimens from patients with HCV infection, but it was detected only in the liver. These results suggest that HCV replicates predominantly in liver cells. The detection of minus-strand HCV RNA should be useful for determining HCV replication in tissues other than liver (Takehara *et al.*, 1992).

1.2.3 *Interpretation of serological markers of HCV infection*

Patients infected with HCV may or may not develop clinical and biochemical evidence of acute hepatitis. First-generation assays may give positive results at the time of acute hepatitis or not for months after acute infection, so repeat testing up to 12 months after onset of disease is necessary before HCV infection can be ruled out as a cause of non-A, non-B hepatitis. Even then, given the limited sensitivity and specificity of first-generation assays, the diagnosis cannot be made with certainty. Second-generation assays usually give positive results at the onset of clinical disease, but repeat testing may be necessary even with these tests. After acute infection, approximately 50% of patients become asymptomatic and have normal transaminase levels (Alter *et al.*, 1992); however, anti-HCV remains and RNA is found by PCR in the majority of cases, suggesting persistent infection.

About 50% of patients with clinical evidence of acute HCV infection develop persistent or fluctuating increases in the level of alanine aminotransferase, and most of them have a

histological picture of chronic active hepatitis in liver biopsy samples, which may progress to cirrhosis. PCR shows that they also retain anti-HCV and HCV RNA. Thus, the results of tests for both anti-HCV and HCV RNA are usually positive in both individuals with active and those with quiescent HCV infection. Current evidence suggests that few patients resolve HCV infection spontaneously (Alter *et al.*, 1992).

Existing immunoglobulin M–anti-HCV tests, although not commercially available, may prove useful in differentiating acute HCV from exacerbations of chronic disease.

1.3 Epidemiology of infection

Specific tests for hepatitis C became available in 1989 (Kuo *et al.*, 1989), although the existence of a virus distinct from viruses A and B that causes post-transfusion hepatitis had been proposed for years previously (Prince *et al.*, 1974; Anon., 1975). Studies in the USA showed that sporadic cases occurred in addition to those associated with blood transfusion (Alter *et al.*, 1982). The availability of specific tests has begun to clarify the epidemiology. A significant number of false-positive results was obtained using early tests with an ELISA to the C100-3 antigen, particularly in the populations of tropical countries; use of the second-generation tests and confirmation by RIBA has provided more reliable estimates of the prevalence of infection. Table 1 shows the prevalences of specific antibody in various populations and the assays used.

Table 1. Community-based studies of seroprevalence to HCV markers

Region	Assay[a]	Age group (years)	No. of people	% with Ab	Comments	Reference
Cameroon	RIBA	16–70	315	9.8	Rate increased with age; excess in women over men	Mencarini *et al.* (1991)
Swaziland	RIBA	16–50	194	1.5		Aceti *et al.* (1992)
Italy	1st-gen. ELISA	20– ≥ 61	812	2.9	Rate increased with age; excess in men over women	Albano *et al.* (1992)
Italy	RIBA-2	30–69	1484	0.87	Higher prevalence in those 40–59 years old	Rapicetta *et al.* (1992)
Spain	1st-gen. ELISA	6 mo.–75	497	0.61	Excess in men over women	Dal-Ré *et al.* (1991)
Peru	RIBA	14–80	2111 (males)	0		Hyams *et al.* (1992)
Yemen	RIBA	3–80	348	2.6	Rate increased with age	Scott *et al.* (1992)
Hong Kong	1st-gen. ELISA	0– > 60	382	0.5		Chan, G.C.B. *et al.* (1992)
Japan	1st-gen. ELISA	> 40	1009	2.3	Rate increased with age; excess in men over women	Ito *et al.* (1991)
USA	RIBA	≥ 15	2523	18.0		Kelen *et al.* (1992)

[a]ELISA, enzyme-linked immunosorbent assay; RIBA, recombinant immunoblot assay: sera were initially screened using first- and second-generation ELISA, and reactive sera were further verified with a second-generation immunoblot assay; RIBA-2, second-generation RIBA, with C22, 5.1.1, C100-3 and C33 antigens (sometimes called RIBA 4)

Screening of blood donors has also provided information on prevalence, although the exclusion of high-risk groups and those with a history of hepatitis makes these populations less representative. The results of a sample of donor surveys are shown in Table 2. Surveys have also been carried out of pregnant women (Table 3).

Table 2. Prevalence of HCV antibodies among blood donors in various regions

Country or region	Assay[a]	Age group (years)	No. of people	% with Ab	Reference
Niger	2nd-gen.	mean, 30	1068 men	0.56	Develoux *et al.* (1992) (abstract)
United Kingdom	RIBA-2	NR	31 936	0.08	Goodrick *et al.* (1992)
Germany	1st-gen.	18–65	116 700	0.72	Caspari *et al.* (1991)
Saudi Arabia	RIBA (5-1-1, C100-3)	NR	4580 Saudis 1694 Middle East 1824 Far East 2548 European/ American	0.33 1.42 0.27 0.27	Bernvil *et al.* (1991)
Kuwait	1st-gen.	NR	505	3.0	Al-Nakib *et al.* (1992)
Thailand	1st-gen.	10–70	390	2.6	Boonmar *et al.* (1990)
Hong Kong	1st-gen.	NR	4291	1.24	Lin *et al.* (1992)
China	RIBA-2	18–50	503	1.6	Zhang *et al.* (1992)
Japan	1st-gen.	15– > 60	2970	1.14	Watanabe *et al.* (1990)
Australia	RIBA-2 or -4	20–60	94 970	0.31	Archer *et al.* (1992)

NR, not reported

[a]RIBA-2, second-generation recombinant immunoblot assay using 5-1-1, C100-3, C33c and C22-3 antigens: a positive reaction is reactivity against any two of the four antigens; 1st-gen., first generation enzyme-linked immunosorbent assay; RIBA-2 or -4, second-generation RIBA with 5-1-1, C100-3, C33c and C22-3 antigens or only the first two: a positive reaction is reactivity against either the two antigens or the four antigens.

All three survey populations show the same pattern of infection, with rates of 1% or lower in Europe and North America when the RIBA is used, and rates of 1–3% in the Middle East and parts of Asia; only in Central Africa are higher rates seen. In all of these surveys, rates increased with age, particularly after the age of 30. The sex ratio varied from a 2:1 excess in men to an excess in women. In the study of blood donors in Australia, there was a peak prevalence in younger adults (30–34 years in each sex) (Archer *et al.*, 1992).

Specific studies of prevalence have also been carried out in groups considered to be at increased risk. These can be divided into those in which parenteral transmission is considered a risk and those in which risk is considered to increase owing to other behaviour patterns.

1.3.1 *Parenteral exposure*

(*a*) *Occupation*

In Japan, all reported 'needle-stick' injuries in staff at one hospital were studied over the period 1981–89 (Kiyosawa *et al.*, 1991). A total of 110 employees received such injuries while

Table 3. Seroprevalence of HCV antibodies in pregnant women in various regions

Country or region	Assay[a]	Age group (years)	No. of people	% with Ab	Reference
Niger	2nd-gen.	mean, 24.3	355	0	Develoux *et al.* (1992) (abstract)
France	RIBA-2	< 20–> 40	1089	North African, 1.9 Black African, 4.8 European, 0 Asian, 1.8	Aussel *et al.* (1991)
France	RIBA-2	17–45	2367	French, 0.99 African, 1.06	Roudot-Thoraval *et al.* (1992)
Spain	1st-gen.	Not reported	241	1.2	Esteban *et al.* (1989)
USA	RIBA-2	13–43	1005	1.8	van Bohman *et al.* (1992)
Thailand	1st-gen.	18–35	212	2.8	Boonmar *et al.* (1990)
Taiwan	RIBA	23–36	944	0.63	Lin *et al.* (1991)

RIBA-2, second-generation recombinant immunoblot assay; 1st-gen., first-generation enzyme-linked immunosorbent assay

treating HCV-seropositive individuals; four developed acute hepatitis, three seroconverted to anti-HCV, and the remainder did not seroconvert to anti-HCV. A study of 456 New York (USA) dentists in 1985–87 (Klein *et al.*, 1991) demonstrated prevalences of anti-HCV (by ELISA confirmed with RIBA) of 1.75% in male and 1.6% in female dentists and 0.14% among blood donors with at least one year of post-graduate education. The only significant association with HCV seropositivity in this study was with oral surgery; the HCV-seropositive dentists reported having treated more AIDS patients, homosexual men, intravenous drug users and haemophiliacs than those who were seronegative. In contrast, 94 dentists in south Wales (United Kingdom) were all found to be seronegative for anti-HCV (Herbert *et al.*, 1992). In Germany, the prevalence of antibodies to HCV (analysed by RIBA) was 0.58% among 1033 hospital employees and 0.24% among blood donor controls (Jochen, 1992). A study of 945 hospital workers in southern Italy found 4.8% to be seropositive, with a seroprevalence of 1.1% of 3575 blood donor controls (De Luca *et al.*, 1991); 576 factory workers from the same area had a 10% seroprevalence (De Luca *et al.*, 1992). In a haemodialysis unit in Italy, 2.5% of staff members were seropositive for antibodies to HCV (Maggi & Petrarulo, 1992).

(*b*) *Bleeding disorders*

The prevalence of HCV antibody in people with haemophilia A or B or von Willebrand's disease, who receive clotting factors, is shown in Table 4. The first-generation assays appeared to be less sensitive than the second-generation assays.

Table 4. Prevalence of antibodies to HCV in people with blood clotting disorders

Country or region	No. of people	Bleeding disorder	Assay[a]	% with Ab	Reference
Spain	97	Haemophilia	1st-gen. RIA	63.9	Esteban *et al.* (1989)
Australia	176	165 with haemophilia A, 5 with haemophilia B, 6 with von Willebrand's disease	1st-gen. ELISA	75.6	Fairley *et al.* (1990)
Germany	28	Haemophilia	ELISA	85.7	Abb (1991)
Sweden	141	112 with haemophilia A, 29 with haemophilia B	1st-gen. ELISA	86.5	Widell *et al.* (1991)
USA	131	117 with haemophilia A, 12 with haemophilia B, 1 asymptomatic haemophila carrier, 1 with von Willebrand's disease	1st-gen. ELISA	76.3	Brettler *et al.* (1990)
Scotland	78	66 with haemophilia A, 19 with haemophilia B	RIBA-2	96.2	Watson *et al.* (1992)
France	42	Haemophilia	EIA-2	100	Laurian *et al.* (1992)
Australia	392	331 with haemophilia A, 40 with haemophila B, 21 with von Willebrand's disease	1st-gen. ELISA	73.0	Leslie *et al.* (1992)

[a]1st-gen. RIA, first-generation radioimmunoassay; 1st-gen. ELISA, first-generation enzyme-linked immunosorbent assay; RIBA-2, second-generation recombinant immunoblot assay; EIA-2, second-generation enzyme immunoassay

(c) *Renal patients*

A number of studies have been carried out in patients undergoing haemodialysis for renal failure or who have received renal transplants (Table 5). These studies show a relationship between HCV seropositivity and previous blood transfusion and duration of haemodialysis. In peritoneally dialysed patients, previous haemodialysis was a significant risk factor for seropositivity; the first-generation assays had a significant rate of false-negativity.

Kidney transplant patients in France had a seroprevalence of antibodies to HCV of 23.6% (Pol *et al.*, 1992). Of 27 patients followed prospectively, 10 (37%) were already seropositive at the time of transplantation and remained anti-HCV seropositive during follow-up, 11 (41%) patients developed antibody at an average of 95 months after renal transplantation, and six initially seropositive patients (22.2%) lost antibody at an average of 111 months after transplantation. In a similar study in Spain, 32 (48%) of 67 patients were seropositive at the time of transplantation, nine of the 32 (28%) lost antibody after transplantation and five of the remaining 35 seronegative patients (14%) became seropositive (Ponz *et al.*, 1991).

(d) *Intravenous drug users*

Studies of seroprevalence for antibodies to HCV among intravenous drug users are summarized in Table 6. Rates of infection are high in all geographical areas, and, when it was

Table 5. Prevalence of antibodies to HCV in patients on renal dialysis

Country or region	Treatment	Assay[a]	No. of people	Prevalence (%)	Reference
New Zealand	Peritoneal dialysis	EIA-2	35	8.6	Blackmore *et al.* (1992)
	Haemodialysis		53	1.9	
	Transplantation		155	4.5	
Germany	Haemodialysis	ELISA-2	498	23.1	Schlipköter *et al.* (1992)
Italy	Peritoneal dialysis	RIBA	64	4.8	Brugnano *et al.* (1992)
	Haemodialysis		205	13.3	
Italy	Haemodialysis	ELISA C100	177	10.2	Fabrizi *et al.* (1992)
Italy	Haemodialysis	RIBA	146	21.9	Maggi & Petrarulo (1992)
Italy	Haemodialysis	ELISA-2	185	38.0	Mosconi *et al.* (1992)
Italy	Haemodialysis	RIBA	318	25.5 (mean of 3)	Vandelli *et al.* (1992)
Saudi Arabia	Haemodialysis	RIBA	66	45.5	Al Nasser *et al.* (1992)
Taiwan	Haemodialysis	EIA-2	125	47.2	Sheu *et al.* (1992a)
China	Peritoneal dialysis	EIA	101	29.7	Ng *et al.* (1991)
Japan	Haemodialysis	RIBA	393	17.8	Tamura *et al.* (1992)
Japan	Haemodialysis	ELISA-3	489	41.9	Fujiyama *et al.* (1992)
Spain	Haemodialysis	RIA	42	19.1	Esteban *et al.* (1989)
Australia	Dialysis (unspecified)	ELISA-2	205	5.9	Fairley *et al.* (1990)
	Renal transplantation		261	6.9	
Germany	Haemodyalisis	ELISA	22	9	Abb (1991)

[a]EIA-2, second-generation enzyme immunoassay; ELISA-2, second-generation enzyme-linked immunosorbent assay; RIBA, recombinant immunoblot assay; ELISA C100, ELISA with C100 antigen

examined, duration of intravenous drug use was found to be significantly associated with HCV seropositivity.

1.3.2 *Non-parenteral exposure*

(*a*) *Perinatal*

Inoue *et al.* (1991) reported on a grandmother, mother and baby in Japan, all of whom were seropositive for amplified HCV DNA fragments. The baby developed clinical hepatitis and was seropositive for HCV antibody (C100) in an ELISA; the mother and grandmother had antibodies to the nucleocapsid P22 antigen. A study of the offspring of 17 HCV antibody-seropositive women in Hong Kong (Reesink *et al.*, 1990) revealed only one seropositive for HCV antibody, although six babies of 217 HCV-seronegative women were seropositive; the difference was not significant. A study of 13 children born to nine HCV antibody-seropositive women in Japan (Kuroki *et al.*, 1991) showed that passively transmitted

Table 6. Prevalence of antibodies to HCV in intravenous drug users

Country	Assay[a]	No. of people	% with Ab	Reference
Spain	1st-gen. RIA	83	71.1	Esteban *et al.* (1989)
Australia	ELISA-2	172	86.0[b]	Bell *et al.* (1990)
Australia	ELISA-2	431	61.9	Fairley *et al.* (1990)
Italy	1st-gen. ELISA	80	67.5	Girardi *et al.* (1990)
Germany	1st-gen. ELISA	51	63	Abb (1991)
Sweden	1st-gen. ELISA	172	80	Widell *et al.* (1991)
Netherlands	1st-gen. ELISA	304	73.7	van den Hoek *et al.* (1990)
USA	RIBA	225	85.3	Donahue *et al.* (1991)
Canada	1st-gen. ELISA	76	50.0	Anand *et al.* (1992)

[a]1st-gen. RIA, first-generation radioimmunoassay; ELISA-2, second-generation enzyme-linked immunosorbent assay; RIBA; recombinant immunoblot assay
[b]Among people injecting drugs for more than eight years, there was 100% anti-HCV seropositivity.

antibody persisted up to six months of age; after that age, all of the babies were seronegative but 11 of the 13 children were HCV RNA seropositive. In two of these mother–child pairs, the mother had been transfused after birth and may have acquired HCV by that route. A further study of eight HCV-seropositive women (Thaler *et al.*, 1991) confirmed that passive antibody was lost by nine months of age, but all of the children were HCV RNA seropositive. No relationship was seen with the human immunodeficiency virus (HIV) status of the mother.

A study of the infants of 43 intravenous drug users (Weintrub *et al.*, 1991), using RIBA, showed that 17 children had passive antibody up to the age of four months. Three of 24 initially seronegative infants were persistently seropositive for antibodies to HCV up to 18 months of age.

In a study in Spain of transmission among HIV-seropositive, HCV-seropositive mothers (Perez Alvarez *et al.*, 1992), 21 of 22 children had maternal HCV antibodies, which became undetectable by three months of age. One child had persistent antibodies and went on to develop non-A, non-B hepatitis with HIV infection. The mother of this child had advanced AIDS. In a study of eight pregnant women with HCV RNA detected by PCR (Novati *et al.*, 1992), five of the women were seropositive for HCV (all were also seropositive for HIV). Four of eight children were seropositive for HCV RNA, three of them at birth. One child had persistent viraemia, and the other three were intermittently seropositive for HCV RNA. All three children lost antibody in the same way as the children who were not viraemic.

(b) Familial and household transmission

As HBV is known to be transmitted within the households of carriers, some workers have examined the prevalence of infection in households of people known to be HCV antibody seropositive. In Spain, Menéndez *et al.* (1991) studied 530 household contacts of 225 subjects seropositive for antibodies to HCV: 26 relatives (4.9%) were seropositive—a

significantly greater proportion than among blood donors. There was no difference in prevalence between sexual and non-sexual contacts. The seroprevalence of HCV antibody in the contacts increased with age and was highly correlated with duration of contact with the index patient. A study of household contacts of seropositive haemodialysis patients in Italy (Calabrese *et al.*, 1991) showed 7% of 30 family members to be seropositive. In a second study in Italy (Mondello *et al.*, 1992), household contacts of patients with cirrhosis were examined, comprising eight husbands, eight wives, 44 children and 57 siblings of 21 patients. Two partners (12.5%), five of the children (11.3%) and 27 of the siblings (48.8%) were seropositive for antibodies to HCV (tested by RIBA-2). These prevalences were similar to those of HBV infection in the same families. In Japan, the seroprevalence of HCV antibody (by ELISA) was zero in a survey of 1442 schoolchildren (Tanaka, E. *et al.*, 1992). In Saudi Arabia (Bahakim *et al.*, 1991), however, marked geographical variation in the seroprevalence of antibody was seen in children under 10 years of age: in Riyadh, 0.9%; in Taif, 1.5%; and in Gizan, 5.7%. A report from Canada (Chaudhary *et al.*, 1992) in a home for the mentally handicapped, showed no HCV antibody (by ELISA-2) in a group of 264 children (128 with Down's syndrome), although there was a high rate of HBV infection.

(*c*) *Sexual transmission*

In two studies, HCV RNA was not detected by PCR in the semen of patients seropositive for HCV antibody (by ELISA) and with chronic hepatitis C (Fried *et al.*, 1992; Terada *et al.*, 1992). In contrast, in a study of 34 patients with chronic hepatitis C who were seropositive for HCV RNA, 24% of seminal fluid samples and 48% of saliva samples contained HCV RNA. Subjects who were seronegative for HCV RNA but seropositive for antibodies to HCV had no HCV RNA in body fluids (Liou *et al.*, 1992). An increased frequency of antibodies to HCV (by ELISA) was found in the semen of non-A, non-B hepatitis patients over that in controls (Kotwal *et al.*, 1992b).

Epidemiological evidence of sexual transmission has been sought by studying the prevalence of infection in sexually active people and in sexual partners of infected individuals. In Canada (Anand *et al.*, 1992), 9.3% of homosexual or bisexual men who were also HIV seropositive were found to be HCV seropositive, compared with 6.4% of a similar group who were HIV seronegative. The difference was not significant. A study of homosexual men in Italy (Gasparini *et al.*, 1991) (using first-generation ELISA) found a seroprevalence of HCV infection of 18.9%, but no association was seen with HIV or HBV seropositivity, with the type of intercourse or with sexual promiscuity. The seroprevalence of hepatitis C was 1.6% in a group of 926 homosexual or bisexual men in Baltimore, USA. Only intravenous drug use and a history of hepatitis A were associated with HCV seropositivity; there was no association with HIV-1 seropositivity or sexual behaviour variables (Donahue *et al.*, 1991). Studies of HCV antibody seroprevalence in people attending clinics for sexually transmitted diseases showed an association with such diseases, which is not as strong as that with HIV-1 or HBV (Corona *et al.*, 1991; Ranger *et al.*, 1991; Gutierrez *et al.*, 1992; Schoub *et al.*, 1992).

Studies of seroprevalence in spouses (using antibody assays) have provided little evidence of sexual transmission (Lin *et al.*, 1991; Chan, G.C.B. *et al.*, 1992). In a study of

195 spouses of Japanese patients with HCV-related chronic liver disease (Akahane *et al.*, 1992), those who had had transfusions or a history of hepatitis before marriage were excluded. The remaining 176 were tested for HCV core antibody, HCV RNA and C100 antibody (by ELISA). Of the spouses, 6% were seropositive for C100, 12% were seropositive for core antigen and seronegative for C100, and 18% were seropositive for both; 8% were HCV RNA seropositive. No controls were used in this study, but genotyping of RNA from six spouse pairs showed concordance in all of them. Studies of HCV RNA require careful interpretation.

(*d*) *Population transmission*

A number of researchers have attempted to determine the modes of transmission in populations. Alter, M.J. *et al.* (1989) carried out a case–control study in which the cases were patients with notified acute non-A, non-B hepatitis in two counties of the USA over a 12-month period. People with a known source of infection—a history within the preceding six months of blood transfusion (13%) or intravenous drug use (34%)—were excluded, leaving 74 cases. Matched controls were selected for 52 (70%) of these cases. No increased risk was found for a range of activities, including homosexual activity, health care employment, surgery, dental work or international travel; however, significant odds ratios were found for individuals with $\leq$ 12 years of education, more than two sexual partners and a history of hepatitis in household or sexual contacts. The last two factors were considered to be responsible for 5% and 6% of all non-A, non-B cases, respectively.

Pohjanpelto (1992) enquired about risk factors for transmission from all individuals found to be seropositive for antibodies to HCV (by enzyme immunoassay) in a laboratory in Finland. Information was obtained for 160 of 276 seropositive individuals, of whom 89% reported exposure *via* blood (64% due to intravenous drug use and 24% due to transfusion), 0.6% had a sexual partner seropositive for HCV antibody, and 8.1% had lived or travelled to countries such as Somalia, Egypt and Saudi Arabia. There were no controls in this study. In Texas, USA (van Bohman *et al.*, 1992), 23 pregnant women seropositive for HCV antibody (by ELISA; 18 with confirmation by RIBA) were compared with seronegative women, giving 1005 consecutive births. Seropositivity was significantly associated with intravenous drug use, a history of sexually transmitted disease, a history of HBV infection, sex with an intravenous drug user and more than three sexual partners during life. In a case–control study on blood donors in Sydney, Australia (Kaldor *et al.*, 1992), the cases were people who were repeatedly seropositive for antibodies to HCV (by ELISA and RIBA), and controls were those repeatedly seropositive by ELISA and seronegative by RIBA. Highly significant, independent associations with seropositivity were found for intravenous drug use, having a tattoo and the number of heterosexual contacts. Blood transfusion was not a significant risk factor in this study.

[The Working Group noted that the parenteral route is a major source of infection in some populations. They also noted that sexual and perinatal transmission can occur but that current data do not allow estimation of their relative importance in different populations.]

1.4. Clinical diseases (other than cancer)

HCV is the major cause of parenterally transmitted non-A, non-B hepatitis worldwide. Exposure to this agent often results in a clinically indolent infection, which, however, carries a risk of long-term morbidity (Mondelli & Colombo, 1991; Czaja, 1992).

1.4.1 *Acute infection*

The time-lag between exposure to HCV during transfusion and development of clinical acute hepatitis is 2–26 weeks, with a peak of onset between 6 and 12 weeks (Alter, H.J. *et al.*, 1989). Using second-generation ELISA, which detects serum antibodies against both structural and non-structural proteins of HCV, the mean time between exposure and seroconversion is 2.3 weeks (Mattsson *et al.*, 1992). In patients with transfusion-associated non-A, non-B hepatitis followed prospectively for 10–14 years, the time between exposure to HCV and onset of hepatic virus replication, detected by serum HCV RNA, is as short as one week (Farci *et al.*, 1991). The hepatitis is clinically mild during its acute phase. The range of serum alanine aminotransferase (ALT) levels is 200–600 IU/L, and 75% of cases are anicteric and relatively asymptomatic (Alter, H.J. *et al.*, 1989; Aach *et al.*, 1991). In contrast, community-acquired hepatitis C is more often symptomatic (Alter *et al.*, 1992). A likely explanation for this discrepancy is that studies of community-acquired hepatitis have as their starting point the enrolment of patients with clinically detectable disease. Thus, the occurrence of community-acquired hepatitis C is probably underestimated because of the large number of subclinical cases that escape detection.

One characteristic feature of hepatitis C is a fluctuating serum ALT pattern (Alter H.J. *et al.*, 1989; Mondelli & Colombo, 1991). Patients may have highly fluctuating ALT levels within periods of time as short as one week, and such variations may persist. A smaller number of patients have a single ALT peak and then proceed to apparent full recovery, or a plateau-like, mild elevation of ALT level. Patients with a monophasic pattern of serum ALT recovered from hepatitis more often than patients with either fluctuating or plateau-like serum ALT patterns (Tateda *et al.*, 1979). A feature of hepatitis in some patients is an apparently long-lasting normalization of serum ALT, suggesting full recovery, which is followed later by symptomless enzymatic exacerbations. Antibody against the non-structural C100-3 epitope of HCV (by first-generation ELISA) disappears from almost all patients who recover clinically and biochemically but persists in patients with chronic hepatitis (Alter, H.J. *et al.*, 1989; Alberti, 1991).

While the majority of cases of acute hepatitis C are clinically indolent, severe cases occur. Fulminant hepatic failure is seen rarely in patients who are immunosuppressed or have pre-existing liver disease. On the basis of serum HCV RNA, a marker for replicating virus, HCV may be the cause of hepatic failure in up to 18% of cases of fulminant non-A, non-B hepatitis (Theilmann *et al.*, 1992; Féray *et al.*, 1993). In some other patients with fulminant hepatitis, HCV has been implicated as a cofactor in conjunction with other hepatitis viruses (HAV, HBV or HDV) (Féray *et al.*, 1993) or with drugs.

1.4.2 *Chronic infection*

Twenty percent of all patients with chronic hepatitis C progress to cirrhosis, regardless of the route of infection (Mendenhall *et al.*, 1991; Mondelli & Colombo, 1991).

In many patients, development of chronic liver disease is heralded by persistent elevations in serum ALT activity for more than six months after the onset of acute hepatitis C, and is accompanied by persistence of serum antibodies to HCV and HCV RNA. Histological features of chronic liver disease and cirrhosis have also been detected, however, in seropositive viraemic patients with a persistently normal ALT level (Alberti *et al.*, 1992). In most patients, progression of hepatitis C to cirrhosis is a clinically indolent process, with an average length of approximately 20 years (Kiyosawa *et al.*, 1990a). Even the apparently benign disease, chronic persistent hepatitis C, entails a risk of progression to cirrhosis (Hay *et al.*, 1985). As with tranfusion-associated infection, most patients with HCV acquired by other routes had persistent infection for several years, even in the absence of active liver disease (Alter *et al.*, 1992).

Chronic HCV infection may have important clinical consequences. In a long-term multicentre follow-up study of 568 patients who developed post-transfusion hepatitis between 1967 and 1980, there was a small but significant increase in the number of deaths related to liver disease (Seeff *et al.*, 1992).

The factors that influence the severity of liver damage and the rate of progression to cirrhosis in patients with HCV infection are largely unknown. Clinical and epidemiological factors that may predict the severity of chronic hepatitis C include age at infection, duration of disease, serum ALT levels, co-occurrence of HBV infection and alcoholism. In haemophiliac patients, chronic hepatitis and cirrhosis were more often detected in those with persistently elevated serum levels of ALT (54%) than in individuals with only intermittently abnormal enzyme levels (7%) (Colombo *et al.*, 1988). In a US multicentre study of alcoholics, anti-HCV was more frequently associated with cirrhosis than with less severe hepatic lesions (Mendenhall *et al.*, 1991). Finally, studies of virus genotyping have indicated that the severity of liver disease correlates well with the predominance of the Japanese strain or with the co-occurrence of multiple strains (Takada *et al.*, 1992).

There is controversy about whether the course of HCV infection is different in immunocompromised patients. In a three-year follow-up of 97 patients with non-A, non-B hepatitis (Martin *et al.*, 1989), there was evolution to cirrhosis in 11, including the only three with HIV infection; these three patients developed symptomatic cirrhosis within three years of the onset of hepatitis. The serum titres of HCV RNA were higher in HIV-seropositive than in HIV-seronegative patients (Wright *et al.*, 1992a). Liver-graft infections recurred in almost all patients who were infected with HCV before transplantation, and accelerated hepatic histological deterioration was seen in some (Féray *et al.*, 1992; Wright *et al.*, 1992b).

1.4.3 *Extrahepatic manifestations*

Several extrahepatic manifestations of HCV infection have been described. For instance, 82% of 74 Italian patients with porphyria cutanea tarda had circulating antibodies to HCV (by RIBA) (Fargion *et al.*, 1992). A serum sickness-like syndrome has been described in patients with acute non-A, non-B hepatitis (Perrillo *et al.*, 1981). The possible link betweem HCV and such extrahepatic syndromes as polyarteritis nodosa and idiopathic pulmonary fibrosis is under debate. Antibodies to HCV were detected (by ELISA-2, confirmed by RIBA-2) in three (8%) of 38 patients with polyarteritis nodosa (Deny *et al.*, 1992). Antibodies to HCV were found in 19/66 (29%) Japanese patients with idiopathic

pulmonary fibrosis by ELISA-1, whether or not there was chronic liver disease; RIBA was used to confirm the presence of antibodies in 12/19 patients (Ueda *et al.*, 1992). HCV has been implicated in many cases of type-II cryoglobulinaemia. Agnello *et al.* (1992) detected HCV RNA in 16/19 such patients and antibody to HCV in eight (by RIBA). Quantitative studies in four patients showed that almost all of the HCV RNA sequences were concentrated in the cryoprecipitate. Eight patients with membranoproliferative glomerulonephritis had circulating antibodies to HCV (by RIBA), and cryoglobulin-like structures, immunoglobulin G and M and C3 antigen were demonstrated within the glomeruli (Johnson *et al.*, 1993). In another study, 57% of 28 patients with chronic hepatitis C had histological evidence of Sjøgren's syndrome, compared with only 5% of controls with miscellaneous diseases (Haddad *et al.*, 1992).

1.5 Therapy

No vaccine is currently available for HCV infection.

1.5.1 *Acute and fulminant HCV infection*

Most cases of acute HCV infection are asymptomatic and do not require medical attention. In cases of malaise and fatigue, bed rest is advised. In symptomatic acute HCV infection, therapy is aimed at relief of the signs and symptoms associated with the acute phase of the disease. It includes parenteral nutrition in cases of dehydration and inanition due to nausea and vomiting, and replacement of coagulation factors in cases of bleeding due to impaired synthetic liver function. While fulminant hepatitis C is a rare clinical entity (Wright *et al.*, 1991; Féray *et al.*, 1993; Liang *et al.*, 1993), liver transplantation is a therapeutic option in advanced liver failure and hepatic coma (Maddrey & Van Thiel, 1988). Few trials of antiviral agents in acute HCV infection have been carried out with the intention of preventing the progression of acute hepatitis C to chronic liver disease. While in a study from Japan, natural β-interferon appeared to be effective (Omata *et al.*, 1991), a study from Spain involving recombinant α-interferon showed no benefit for the long-term outcome of the disease (Viladomiu *et al.*, 1992).

1.5.2 *Chronic HCV infection*

Because of the potentially severe natural course of HCV infection, several therapeutic strategies have been explored. While ribavirin therapy did not significantly affect HCV replication (Di Bisceglie *et al.*, 1992), administration of α-interferon three times per week (Davis *et al.*, 1989; Di Bisceglie *et al.*, 1989; Ruiz-Moreno *et al.*, 1992; Shindo *et al.*, 1992a) or continuously (Carreño *et al.*, 1992) was effective in about 50% of patients, resulting in normalization of liver function, disappearance of HCV RNA from serum and improved liver histology. Unfortunately, about 50% of patients suffer a relapse after cessation of therapy, with increased serum transaminase levels and reappearance of HCV RNA (Davis *et al.*, 1989; Di Bisceglie *et al.*, 1989; Carreño *et al.*, 1992; Garson *et al.*, 1992a; Ruiz-Moreno *et al.*, 1992; Shindo *et al.*, 1992a). A long-term response to α-interferon therapy therefore occurs in only about 25% of patients with chronic HCV infection. Studies are under way to explore the benefit of long-term therapy of chronic HCV infection with α-interferon.

The parameters that predict a response to α-interferon therapy are not well defined (Black & Peters, 1992). In contrast to HBV infection, co-infection with HCV and HIV does not seem to reduce the efficacy of α-interferon therapy (Boyer *et al.*, 1992), but patients immunosuppressed after organ transplantation respond poorly to α-interferon therapy (Davis, 1989; Wright *et al.*, 1992). Recent evidence suggests that response to α-interferon therapy may be related to the viral genotype and viral load in a given individual with HCV infection (Kanai *et al.*, 1992; Yoshioka *et al.*, 1992). The issue is further complicated by the fact that different genotypes have been identified within individuals (Martell *et al.*, 1992; Murakawa *et al.*, 1992; Tanaka T., *et al.*, 1992; Weiner *et al.*, 1992).