Int. J. Exp. Path. (1994) 75, 61–69

Current Status Review

The clinical virology of hepatitis C virus

WILLIAM L. IRVING

Department of Microbiology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH, UK

The continued occurrence of post-transfusion hepatitis (PTH) even after the introduction of diagnostic assays for hepatitis A and B viruses in the 1970s and the exclusion of HBV carriers from the blood supply, led to the recognition of the syndrome of parenterally-transmitted non-A non-B hepatitis (NANBH). The search for the viral agent(s) responsible for this form of NANBH became something of a quest for the Holy Grail for clinical and molecular virologists. After several false dawns, scientists from the Chiron Corporation in California announced the identification of a virus, now known as hepatitis C virus (HCV), which, on serological grounds, was shown to be responsible for the vast majority of post-transfusion NANBH (Choo *et al.* 1989; Kuo *et al.* 1989). The strategy which finally produced this breakthrough was based on the cloning of nucleic acids derived from highly infectious chimpanzee plasma, and screening the library of clones so produced with a human chronic NANBH serum. This represents a major success story for molecular biological techniques in characterizing novel human pathogens.

In the short time that has elapsed since the first description of HCV, knowledge of the molecular and clinical virology of this virus has expanded exponentially. This review is intended to summarize current information on this clinically important virus.

The virus

HCV is a positive strand RNA virus, distantly related to the pestiviruses and flaviviruses (Miller & Purcell 1990). The genomic structure of HCV is illustrated in Figure 1. A non-coding region at the 5′ end (5′NCR) of approximately 332 nucleotides is followed by a continuous open reading frame (ORF) encoding a polyprotein of around 3010 amino acids, and then a short 3′NCR. By analogy with the genomic organization of the pestiviruses and flaviviruses, the 5′ end of the ORF encodes the putative core (C) and envelope (E1 and E2) (i.e. structural) proteins, whilst the remainder of the ORF encodes the non-structural proteins (NS1–5) (Choo *et al.* 1991). The NS3 gene encodes a protease enzyme, one function of which is cleavage of the polyprotein into the individual protein components of the virus, whilst the NS5 gene encodes the viral RNA polymerase enzyme.

The entire nucleotide sequence is available for a number of isolates of HCV, and partial sequences for many more. Some of these sequences show considerable genomic heterogeneity (e.g. only 69% genome homology), leading to the realization that HCV exists as a number of distinct genotypes. The nomenclature for these genotypes has been extremely confusing. However, a new classification system has recently been proposed, which currently recognizes six distinct genotypes, and a number of subtypes (Simmonds *et al.* 1993a) (Table 1). The geographic distribution of these types is uneven. Types 1–3 account for all infections in Scotland, Finland, The Netherlands, Australia and Japan. Type 4 is highly prevalent in Egypt and has also been described in Zaire, suggesting a broad distribution in Africa. Type 5 has been reported in South Africa (Simmonds *et al.* 1993a).

The existence of multiple genotypes of HCV has a number of implications, not the least being in vaccine development. Different regions of the genome show differing degrees of variability, e.g. the NS4 gene is less well conserved than the core gene which in turn shows more variability than the 5′NCR. A putative vaccine may have to contain either highly conserved epitopes, or multiple versions of the same epitope representing all

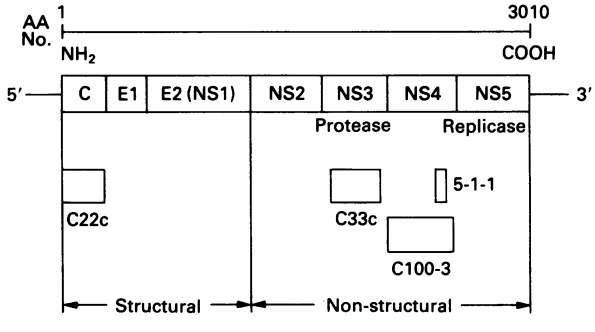

Figure 1. Schematic representation of the hepatitis C virus genome. C22c, C33c, C100–3, and 5-1-1 refer to the recombinant antigens present in second generation assays (see text). 5-1-1 was the first clone identified.

Table 1. Proposed nomenclature for HCV genotypes

Proposed name	HCV variant
1a	HCV-1 HCV-H
1b	HCV-J HCV-BK
1c	EG-28
2a	HC-J6
2b	HC-J8
2c	TO994
3a	E-b1 Ta
3b	Tb
4	EG-16, 29, 33
5	SA-1, 7, 11
6	HK-1, 2, 3, 4

the type-specific variations, to be maximally effective. There is also considerable interest in the relative pathogenicity of the different genotypes and in their relative sensitivity to antiviral agents. Preliminary data suggest that patients infected with different genotypes may respond differently to interferon therapy (Kanai *et al.* 1992).

Diagnostic assays

There are essentially two approaches to the diagnosis of HCV infection: the demonstration of specific antibody, and the detection of the viral genome. Further advances in HCV diagnosis have concentrated on methods designed to identify the genotype(s) of HCV infecting a particular patient.

Serology

First, second, and third generation ELISAs. The first HCV clone to be identified contained viral cDNA derived from the NS4 gene. The antigen encoded by this sequence was known as 5-1-1. An overlapping clone, encoding a larger antigen, c100-3, derived from this, was expressed in a yeast vector as a fusion protein attached to the N-terminal end of human superoxide dismutase and was used as antigen in the so-called first-generation serological assays for detection of anti-HCV (Kuo *et al.* 1989). Whilst these assays were a major advance in the diagnosis of HCV infection, they were of poor sensitivity and specificity, especially in low-risk populations, and were soon replaced by second-generation assays. These assays included recombinant or synthetic peptide antigens derived from both structural and non-structural genes (Figure 1), with much improved sensitivity. Efforts to improve assay performance are resulting in the emergence of third-generation assays. These have two potential advantages: the addition of antigens derived from the NS5 gene, and the use of multiple peptide antigens in place of some of the recombinant proteins.

Confirmatory assays. In general, the confirmatory serological assays have used the same antigens present in the ELISAs but presented in a different format. The most widely used confirmatory assay, the second generation recombinant immunoblot assay (RIBA), uses the c22, c33c, c100 and 5-1-1 recombinant antigens impregnated as bands in a strip of nitrocellulose, together with a superoxide dismutase band and weak and strong positive controls. The efficiency of the washing steps is improved compared to microtitre plates and this is believed to be the basis for the better specificity of this assay. Sera are deemed to be anti-HCV positive if any two (or more) antigen bands are produced, negative if no bands are produced, and indeterminate if only a single band appears. However, recent data suggest that those sera whose RIBA positivity consists of 5-1-1 and c100 antigen bands only, are very unlikely to be infected with HCV (Irving *et al*, 1993a). A third-generation RIBA, modified as for the third-generation ELISA, and also with the 5-1-1 and c100 antigens combined into a single band, will shortly be commercially available. Alternative confirmatory assays similar in format to the RIBA contain a variety of synthetic peptide antigenic bands ('line' assays), or have antigens electrophoresed before transfer to the nitrocellulose (Western blot assay).

Genome detection assays

The reverse transcriptase/polymerase chain reaction (RT/PCR) is an exquisitely sensitive technique which allows demonstration of the presence of viral RNA in clinical material. Use of this assay has shown that the vast majority of second-generation confirmed anti-HCV positive individuals are chronically infected with HCV, as HCV RNA is present in their sera. A plethora of RT/PCR assays for the detection of HCV RNA have been published (Garson *et al.* 1990) and differ in a number of important details (e.g. method of nucleic acid extraction, which oligonucleotide primers are used, method of visualization of the amplified product). A cautionary note has been struck by a recent report of a quality control exercise in HCV RT/PCR. Only a minority of laboratories

correctly identified a panel of 22 positive and negative sera (Zaaijer *et al.* 1993). Thus, until RT/PCR protocols become more standardized and better controlled, results from different laboratories will not necessarily be comparable.

RT/PCR is a more sensitive assay for diagnosis of HCV infection than the currently available serological ones. This is evidenced by the identification of RT/PCR positive individuals who are antibody negative (Sugitani *et al.* 1992). Presumably, antibodies present in these individuals do not recognize the particular antigens present in the serological assays. This is obviously a major drawback in the attempt to prevent transfusion-transmitted HCV infection by screening all blood donors for the presence of anti-HCV.

HCV genotyping assays

An awareness that HCV exists as a number of distinct genotypes has led to the development of assays designed to identify the specific genotype of HCV infecting particular patients. These include RT/PCR assays using type-specific oligonucleotide primers, RT/PCR amplification of a fragment of the genome followed by digestion of the product with pairs of restriction endonucleases, and the use of type-specific peptide antigens in an ELISA format. The first-mentioned technique utilizes a nested format with first-round amplification using universal primers followed by second-round amplification using a universal sense primer plus a set of anti-sense type-specific primers designed to produce products of differing lengths (Okamoto *et al.* 1992). The second technique uses universal primers to generate an amplified product, the internal sequence of which varies between different types. Thus digestion with certain endonucleases will yield different fragment patterns which differ according to the nature of the genotypes present (McOmish *et al.* 1993). The final technique was developed by identifying type-specific antigenic regions within the NS4 gene using overlapping octapeptides covering the NS4 gene, thus enabling synthesis of type-specific peptides for use in an ELISA (Simmonds *et al.* 1993b). This therefore differs from the other two assays in being a serological technique rather than a genome-based one.

Epidemiology

Routes of transmission

Whilst transmission of NANBH via transfusion of blood or blood products has long been recognized, this cannot represent the natural means by which the virus maintains itself in the population. The natural route of transmission remains unknown. HCV RNA has been detected in the saliva of some anti-HCV positive individuals (Wang *et al.* 1992), and Dusheiko and colleagues (1990) described a case of HCV infection acquired from a human bite. This route may thus be one method of hidden parenteral and person-to-person spread of the virus.

A number of studies have reported elevated seropositivity rates in the sexual partners of HCV-infected individuals, but there is some disagreement as to the extent of sexual spread. Rates up to 24% have been found in partners who have no other risk factor for acquisition of HCV infection. RT/PCR has been used in some of these studies to confirm the serology (Akahane *et al.* 1992; Kao *et al.* 1992). The risk of sexual transmission may be dependent on the degree of liver disease in the index case (Peano *et al.* 1993). There is some dispute as to whether homosexual intercourse is a risk factor for HCV infection with some, but not all, investigators reporting increased seroprevalence rates in homosexuals. Vertical transmission, from mother to baby, although documented (Inoue *et al.* 1991), is also the exception rather than the rule. Recent studies of the offspring of carrier mothers showed in total only 1/45 infants had definite evidence of infection (Reinus *et al.* 1992; Wejstal *et al.* 1992). However, vertical transmission has been reported to be much more frequent in mothers who are also infected with HIV, and the offspring of these mothers may be RT/PCR positive without any evidence of a serological response to infection (Thaler *et al*, 1991).

The parenteral route remains the best characterized and accounts for the high seroprevalence rates seen in intravenous drug abusers (IVDA), haemophiliacs and other recipients of blood and blood products, and haemodialysis patients. The relative importance of needlestick injury as a route of transmission has yet to be defined. A recent case control study looking at risk factors for acquisition of HCV amongst UK blood donors revealed a statistically significant fivefold increased risk of infection amongst health care workers (HCW) (K.R. Neal personal communication), and others have demonstrated increased HCV seroprevalence in different groups of HCW, including dentists (Klein *et al.* 1991). The presumed route of acquisition in these cases is via needlestick exposure. The risk of transmission from an individual needlestick incident from a known HCV-infected patient is of the order of 5–10% (Mitsui *et al.* 1992).

Possible transmission of HCV infection from transplant donor to recipient has been a recent cause for concern and has led to the routine screening of donors for evidence of HCV infection. Immunosuppressed organ

transplant recipients may fail to seroconvert to HCV, leading to an underestimate of the risk of donor-to-recipient transmission if only anti-HCV, and not HCV RNA, is sought in the recipient as evidence of infection (Pereira *et al.* 1992).

Seroprevalence studies

Anti-HCV ELISA screening of blood donors in the UK has yielded a positivity rate of around 0·5–1%. However, the poor specificity of even the second-generation ELISAs in low risk populations is demonstrated by the observation that only a small minority of ELISA positive donors are confirmed as RIBA positive, giving a true seroprevalence rate of the order of 0·1% (Follett *et al.* 1991). This figure is remarkably similar to that reported from a number of other countries that have introduced blood donor screening unlike the situation with HBV where there are wide variations in the prevalence of HBsAg carriage throughout the world. The only country so far reporting a significantly higher rate is Egypt, where the prevalence rate is 10–20%. This extraordinary rate is not due to cross-reactivity, e.g. with other flaviviruses, as it has been confirmed by RT/PCR studies (Saeed *et al.* 1991).

The expected high seroprevalence rates in well characterized high risk groups for NANBH referred to above (i.e. IVDA, haemophiliacs) have been confirmed with the advent of HCV-specific diagnostic assays. Seroprevalence rates on haemodialysis units vary enormously, with reports of between 0 and 30% antibody positivity (Daporto *et al.* 1992). Seroprevalence studies in homosexuals and in the offspring of carrier mothers have been referred to above.

Pathogenesis of disease

The pathogenetic mechanisms whereby HCV infection leads to disease are poorly understood. Virus can infect and replicate within hepatocytes. However, whether liver cell death is due to virus-induced cytolysis or to an immunopathogenic destruction, for example by cytotoxic T cells, has not been resolved. The role of cofactors in inducing liver damage is also unclear. An epidemiological study has suggested that the effects of alcohol and HCV in inducing cirrhosis are additive rather than synergistic (Corrao *et al*, 1992). A number of patients coinfected with both HBV and HCV have been identified. The consequences of this coinfection in terms of risk of liver damage have not been characterized. The association of HCV infection with the development of hepatocellular carcinoma (HCC) is most likely due to the ability of HCV to induce hepatic cirrhosis and the higher risk of HCC developing in cirrhotic liver rather than any tumorigenic property of the virus itself (Simonetti *et al*, 1992). The virus possesses no known reverse transcriptase activity and there is no evidence for a DNA form of the genome which might integrate into the host genome.

HCV RNA has also been detected in peripheral blood mononuclear cells (PBMC) of some anti-HCV positive individuals. The presence of both positive and negative-stranded RNA, together with expression of core-derived antigens in these cells, indicates that PBMC are an additional source of viral replication (Bouffard *et al.* 1992). The pathogenic significance of this extra-hepatic site of viral infection is unclear, although one instance where this may be of importance is in patients undergoing liver transplantation because of end-stage HCV-induced liver disease. Reinfection of the grafted liver has been reported (Bealli *et al.* 1993), and this presumably arises from virus in circulating white cells.

The possible role of autoimmune reactions triggered by HCV infection in inducing a variety of clinical manifestations of infection is of considerable interest (see below).

Clinical consequences of HCV infection (Table 2)

A number of manifestations of infection with HCV are eminently predictable, based on knowledge of the clinical features of NANBH. However, application of diagnostic assays for HCV infection beyond the obvious patient groups has led to a number of unexpected disease associations. Whilst not all of these reports have withstood more detailed investigation, some have, and there is no doubt that the disease spectrum induced by HCV infection is much broader than was originally imagined.

Clearance of infection

Using second-generation assays, a small minority (e.g. 10%) of anti-HCV positive blood donors are persistently RT/PCR negative, even when those with only a 5-1-1/c100 pattern of RIBA reactivity are excluded. These donors may be infected with a type of virus whose sequence is not recognized by the RT/PCR primers or they may have a low-level viraemia below the limit of sensitivity of the assay. A third explanation, which is gaining credence, is that these individuals may have overcome their HCV infection and eliminated the virus. The introduction of a more sensitive third-generation RIBA is resulting in identification of increasing numbers of these individuals, as sera that were previously classified as second-generation RIBA indeterminate are being reclassified as

Table 2. Consequences of infection with HCV

Expected on basis of knowledge of NANBH
Clearance of infection
Unknown percentage of all infections
Asymptomatic carriage
Usually associated with biopsy changes of chronic hepatitis
Chronic hepatitis and cirrhosis
Co-factors increasing risk of progressive disease unknown
Hepatocellular carcinoma
Co-factors unknown. May be consequence of virus-induced cirrhosis
Unexpected
Auto-immune chronic active hepatitis
Especially type 2 (anti-LKM-1 positive). Geographical variation in aetiological importance
Mixed essential (type II) cryoglobulinaemia
Membranoproliferative glomerulonephritis
Porphyria cutanea tarda
Unconfirmed
Sjögren's syndrome
Idiopathic pulmonary fibrosis
Polyarteritis nodosa

RIBA positive, with multiple reactive bands, on the improved RIBA. Many of these sera are RT/PCR negative. The percentage of RIBA positive sera that are RT/PCR positive will therefore decline with the widespread adoption of the more sensitive RIBA and at present it is not possible to state accurately what percentage of HCV-infected blood donors may have cleared the infection. The existence of these individuals is of considerable importance, as an understanding of the mechanisms underlying their ability to clear the virus would be of great interest.

Asymptomatic carriage

Recognition of this possibility came with the development of RT/PCR assays and the subsequent demonstration that the considerable majority of anti-HCV positive individuals, whether suffering from symptomatic liver disease or not, had HCV RNA in their sera. The long-term clinical consequences of this carriage are unclear. Liver biopsies on asymptomatic RT/PCR positive blood donors almost without exception reveal underlying liver damage, the severity covering the whole gamut between mild chronic persistent hepatitis through to cirrhosis (Alberti *et al.* 1992). Liver function tests (e.g. alanine aminotransferase levels) correlate poorly with biopsy appearance and many donors have normal ALT values despite evident liver disease. Similar observations have been reported in patients with sporadic, community-acquired HCV infection followed over a period of 10 years (Alter *et al.* 1992). Despite the disturbing biopsy appearances, there is uncertainty as to the frequency of progression to clinically symptomatic and life-threatening liver disease. A study documenting long-term mortality after transfusion-associated NANBH, in which 18-year follow-up data were available on over 500 cases and controls, showed no increase in mortality from all causes in the NANBH group and only a small increase in the number of deaths related to liver disease (Seeff *et al.* 1992).

Chronic hepatitis and cirrhosis

There is no doubt that chronic HCV infection can result in symptomatic liver disease leading ultimately to liver failure. Surveys of patients who presented with chronic NANBH hepatitis and cirrhosis, whether associated with transfusion or not, and of patients labelled as cryptogenic cirrhosis, have shown high seropositivity rates for HCV, strongly implicating HCV as the cause of these diseases (Bruix *et al.* 1989; Diodati *et al.* 1991). The risk to an individual patient infected with HCV of developing these complications, and the role of possible cofactors in enhancing this risk, are unknown.

Hepatocellular carcinoma, HCC

Several lines of evidence implicate HCV infection as a predisposing factor in the development of HCC. There are case reports documenting the progression of disease in individual patients from post-transfusion hepatitis through to HCC over a number of years (e.g. Tremolada *et al.* 1990). Surveys of patients with HCC and appropriate controls have demonstrated high seropositivity rates for HCV in the patient groups, although the relative risk differs between geographically distinct studies (Bruix *et al.* 1989; Simonetti *et al.* 1992). Patients with HCC have been shown to be RT/PCR positive for HCV RNA (Garson *et al.* 1992), and the viral genome has been demonstrated in the malignant tissue. Little is known of the pathogenesis of the malignant change. Once again, the risk to individual patients, and the role of cofactors, remain to be determined.

Autoimmune chronic active hepatitis. AI-CAH

A possible link between HCV infection and AI-CAH was first suggested by serological data, derived using first-generation assays, purporting to show that most AI-CAH patients were anti-HCV positive. These data were contentious, given the poor specificity of the anti-HCV

ELISAs, and the demonstration that anti-HCV positivity was related to the hyper-gammaglobulinaemia present in these patients (McFarlane *et al.* 1990). The proponents of the original data nevertheless have demonstrated conclusively, with second-generation assays and RT/PCR confirmation, that, in Italy at least, many patients with type 2 AI-CAH (characterized by the presence of anti-liver–kidney–microsome-1 antibodies) are indeed infected with HCV (Garson *et al.* 1991). There appears to be a genuine geographical difference, as yet unexplained, in this phenomenon, as not even type 2 AI-CAH patients in the UK are anti-HCV positive (Lenzi *et al.* 1991).

Further interest in HCV and autoimmunity arises from the discovery of antibodies to an autoantigen, known as GOR, in a high proportion of sera from anti-HCV positive individuals. Whilst attempting to clone HCV from infectious human plasma, Mishiro and colleagues (1990) identified a clone (GOR 47-1) which encoded an antigen to which most NANBH patients had antibodies. However, the nucleotide sequence of this clone was distinct from that of HCV, and in fact GOR is encoded by a host gene (Mishiro *et al.* 1990). The presence of anti-GOR is a remarkably good surrogate marker for HCV infection, and some anti-GOR positive, anti-HCV negative (first-generation assay) sera were shown to be RT/PCR positive. In view of the known occurrence of anti-HCV negative, RT/PCR positive (and therefore presumably infectious) blood donors, it was suggested that the blood supply should be screened for anti-GOR, in addition to anti-HCV. Subsequent studies comparing anti-GOR with second-generation anti-HCV assays have shown that this is unlikely to be of much benefit in the UK (Irving *et al.* 1993b). The nature, function and distribution of the GOR antigen are not known. Moderate sequence homology between part of the core gene of HCV and GOR suggest cross-reactivity as the underlying mechanism of induction of this autoantigen (Hosein *et al.* 1992). The association of HCV infection and manifestations of autoimmune disease is of more than academic importance. Viruses are often implicated as triggers for the development of autoimmunity and HCV may be the best example of possible virus-induced human autoimmunity currently available for further study. In addition, it is clinically important to distinguish classical AI-CAH from that variant associated with HCV infection since optimal treatment of the former is with steroids whilst the latter may respond better to interferon therapy.

Mixed essential (type II) cryoglobulinaemia (MEC)

An aetiological role for hepatotropic viruses in this condition has been suspected because of the high prevalence of coexisting hepatocellular abnormalities in patients with MEC. Several laboratories have reported an increased seroprevalence of anti-HCV in these patients (Pascual *et al.* 1990). The evidence implicating HCV in the pathogenesis of MEC has been strengthened by the demonstration that HCV virions and HCV antigen–antibody complexes are concentrated in the patients' cryoprecipitates (Agnello *et al.* 1992).

Membranoproliferative glomerulonephritis

There have been reports of patients with membranoproliferative glomerulonephritis in the context of HCV-associated mixed cryoglobulinaemia, in whom HCV RNA has been demonstrated in kidney biopsy samples suggesting a direct role for HCV in this disease (Doutrelepont *et al.* 1993).

Porphyria cutanea tarda

As with MEC, the frequent occurrence of liver disease in patients with porphyria cutanea tarda has led to studies of the role of HCV in this disease. In one such study, over 75% of patients with sporadic porphyria cutanea tarda were anti-HCV positive by second generation ELISA and RIBA testing, compared to 0/5 patients with the familial form of the disease. All 18 patients tested by RT/PCR were positive, suggesting that HCV infection may be a trigger for this disease (Herrero *et al.* 1993).

Miscellaneous diseases

Many patients with HCV-associated chronic liver disease were noted to have clinical and labial salivary gland biopsy evidence of Sjögren's syndrome (Haddad *et al*, 1992). However, studies of patients presenting with Sjögren's syndrome in the absence of chronic liver disease have shown no evidence that HCV is a cause of this disease (Aceti *et al.* 1992). One report based on first-generation serology suggested that HCV infection may result in cryptogenic fibrosing alveolitis (Ueda *et al.* 1992). Whilst an intriguing suggestion, implying that the same virus could induce hepatic fibrosis in some individuals but pulmonary fibrosis in others, this observation has not been substantiated using second-generation assays and RT/PCR (Irving *et al.* 1993). A possible role for HCV infection in causing polyarteritis nodosa has been suggested on the basis of a seroprevalence rate of anti-HCV of 12% in PAN patients, using second-generation assays (Cacoub *et al.* 1992).

Antiviral therapy

Interferon alpha has been shown to be effective in treating chronic NANBH, and this promise has been borne out in a number of studies of laboratory proven chronic hepatitis C (e.g. Davis *et al.* 1989). However, response rates (as judged by normalization of ALT values, and/or liver histology) are of the order of only 40–50%, and up to half of the responders relapse when the IFN is stopped. Quantitative RT/PCR studies demonstrate a correlation between clinical response and loss of HCV RNA from serum (Brillanti *et al.* 1991). The return of RT/PCR positivity may be the first indication of relapse following cessation of IFN therapy (Kleter *et al.* 1993). A trial of oral ribavirin showed a decrease in ALT levels whilst patients were on treatment, but not to below the upper limit of normal. ALT levels rose when ribavirin therapy ceased (Reichard *et al.* 1991). Liver transplantation has been successfully used for acute fulminant as well as chronic hepatitis C, but infection can recur in the graft (Belli *et al.* 1993). There is thus considerable need for new and more efficacious forms of therapy for chronic HCV infection.

Given the limitations of the current therapeutic armoury, the management of asymptomatic HCV carriers presents a dilemma; should these individuals be given IFN, even though they may have normal liver function tests? IFN is expensive, not without side-effects, and of limited benefit. There is evidence to suggest that response to IFN may be better the earlier in the disease process it is given (Omata *et al.* 1991), which would argue in favour of treatment at an asymptomatic stage. The answer to this question will become apparent only when the risks of progression to clinically debilitating disease are known.

Future prospects

Despite the explosion in our knowledge of the biology of HCV, there are still a large number of important unanswered questions. What is the natural route of transmission of the virus? What is the natural history of infection in asymptomatic carriers (1 in 1000 blood donors in the UK)? What are the pathogenetic mechanisms underlying HCV-induced disease, in particular those autoimmune manifestations alluded to above? What are the virus or host factors that contribute to the wide variation in disease expression between individuals? Are there any more unexpected disease associations to be uncovered? What alternative forms of therapy will be developed? If our understanding of HCV proceeds at the same rate as it has since the first description of the virus, then the answers to these questions will be forthcoming in the next few years. However, no doubt by then, many more questions will have been raised.

References

Aceti A., Taliani G., Sorice M. & Amendolea M.A. (1992) HCV and Sjögren's syndrome. *Lancet* **339,** 1425–1426.

Agnello V., Chung R.T. & Kaplan L.M. (1992) A role for hepatitis C virus infection in type II cryoglobulinaemia. *New Engl. J. Med.* **327,** 1490–1495.

Akahane Y., Aikawa T., Sugai Y., Tsuda F., Okamoto H. & Mishiro S. (1992) Transmission of HCIV between spouses. *Lancet* **339,** 1059–1060.

Alberti A., Morsica G., Chemello L., Cavalletto D., Noventa F., Pontisso P. & Ruol A. (1992) Hepatitis C viraemia and liver disease in symptom-free individuals with anti-HCV. *Lancet* **340,** 697–698.

Alter M.J., Margolis H.S., Krawczynski K., Judson F.N., Mares A., Alexander W.J., Hu P.Y., Miller J.K., Gerber M.A., Sampliner R.E., Meeks E.L. & Beach M.J. For the Sentinel Counties Chronic Non-A, Non-B Hepatitis Study Team (1992) The natural history of community-acquired hepatitis C in the United States. *New Engl. J. Med.* **327,** 1899–1905.

Belli L.S., Alberti A., Rondinara G.F., De Carlis L., Romani F., Ideo G. & Belli L. (1993) Recurrent HCV hepatitis after liver transplantation. *Lancet* **341,** 378–379.

Bouffard P., Hayashi P.H., Acevedo R., Levy N. & Zeldis J.B. (1992) Hepatitis C virus is detected in a monocyte/macrophage subpopulation of peripheral blood mononuclear cells of infected patients. *J. Infect. Dis.* **166,** 1276–1280.

Brillanti S., Garson J.A., Tuke P.W., Ring C., Briggs M., Masci C., Miglioli M., Barbara L. & Tedder R.S. (1991) Effect of alpha-interferon therapy on hepatitis C viraemia in community-acquired chronic non-A, non-B hepatitis: a quantitative polymerase chain reaction study. *J. Med. Virol.* **34,** 136–141.

Bruix J., Barrera J.M., Calvert X., Ercilla G., Costa J., Sanchez-Tapias J.M., Ventura M., Vall M., Bruguera M., Bru C., Castillo R. & Rodes J. (1989) Prevalence of antibodies to hepatitis C virus in Spanish patient with hepatocellular carcinoma and hepatic cirrhosis. *Lancet* **334,** 1004–1008.

Cacoub P., Lunel-Fabiani F. & Du L.T.H. (1992) Polyarteritis nodosa and hepatitis C virus infection. *Ann. Int. Med.* **116,** 605.

Choo Q.L., Kuo G., Weiner A.J., Overby L.R., Bradley D.W. & Houghton M. (1989) Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* **244,** 359–362.

Choo Q.L., Richman K.H., Han J.H., Berger K., Lee C., Dong C., Gallegos C., Coit D., Medina Selby R., Barr P.J., Weiner A.J., Bradley D.W., Kuo G. & Houghton M. (1991) Genetic organisation and diversity of the hepatitis C virus. *Proc. Natl. Acad. Sci. USA* **88,** 2451–2455.

Corrao G., Carle F., Lepore A.R., Zepponi E., Galatola G. & Di Orio F. (1992) Interaction between alcohol consumption and positivity for antibodies to hepatitis C virus on the risk of liver cirrhosis: a case-control study. *Eur. J. Epidemiol.* **8,** 634–639.

Daporto A., Adami A., Susanna F., Calzavara P., Poli P., Castelletto M.R., Amici G.P., Teodori T. & Okolicsanyi L. (1992) Hepatitis C virus in dialysis units—a multicentre study. *Nephron* **61,** 309–310.

Davis G.L., Balart L.A., Schiff E.R., Lindsay K., Bodenheimer H.C., Perrillo R.P. *et al.* and the Hepatitis Interventional Therapy Group. (1989) Treatment of chronic hepatitis C with

recombinant interferon alpha: a multicentre randomised controlled trial. *New Engl. J. Med.* **321,** 1501–1506.

Diodati G., Tagger A., Bonetti P., Ribero M.L., Drago C., Cavalletto D., Grossi A., Realdi G., Ruol A. & Alberti A. (1991) Antibody to hepatitis C virus in cryptogenic chronic liver disease. *J. Med. Virol.* **35,** 151–154.

Doutrelepont J-M., Adler M., Willems M., Durez P. & Yap S-H. (1993) Hepatitis C infection and membranoproliferative glomerulonephritis. *Lancet* **341,** 317.

Dusheiko G.M., Smith M. & Scheuer P.J. (1990) Hepatitis C virus transmitted by human bite. *Lancet* **336,** 503–504.

Follett E.A.C., Dow B.C., McOmish F., Yap P.L., Hughes W., Mitchell R. & Simmonds P. (1991) HCV confirmatory testing of blood donors. *Lancet* **338,** 1024.

Garson J.A., Lenzi M., Ring C., Cassani F., Ballardini G., Briggs M., Tedder R.S. & Bianchi F.B. (1991) Hepatitis C viraemia in adults with type 2 autoimmune hepatitis. *J. Med. Virol.* **34,** 223–226.

Garson J.A., Ring C., Tuke P. & Tedder R.S. (1990) Enhanced detection by PCR of hepatitis C virus RNA. *Lancet* **336,** 878–879.

Garson J.A., Wicki A.N., Ring C.J.A., Joller H., Zala G., Schmid M. & Buehler H. (1992) Detection of hepatitis C viraemia in Caucasian patients with hepatocellular carcinoma. *J. Med. Virol.* **38,** 152–156.

Haddad J, Deny P., Munz-Gotheil C., Ambrosini J-C., Trinchet J-C., Pateron D., Mal F., Callard P. & Beaugrand M. (1992) Lymphocytic sialadenitis of Sjogren's syndrome associated with chronic hepatitis C virus liver disease. *Lancet* **339,** 321–323.

Herrero C., Vicente A., Bruguera M., Ercilla M.G., Barrera J.M., Vidal J., Teres J., Mascaro J.M. & Rodes J. (1993) Is hepatitis C virus a trigger of porphyria cutanea tarda? *Lancet* **341,** 788–789.

Hosein B., Fang X. & Wang C.Y. (1992) Anti-HCV, anti-GOR, and autoimmunity. *Lancet* **339,** 871.

Inoue Y., Miyamura T., Unayama T., Takahashi K. & Saito I. (1991) Maternal transfer of HCV. *Nature* **353,** 609.

Irving W.L., Day S., Eglin R.P., Bennett D.P., Neal K.R., Jones D.A. & James V. (1993a) Hepatitis C in blood donors. *Lancet* **341,** 835–836.

Irving W.L., Day S., Eglin R.P., Bennett D.P., Nuttall P., Jones D.A. & James V. (1993b) The use of anti-GOR testing in the screening of blood donors for hepatitis C virus infection. *Vox Sang.* **65,** 38–41.

Irving W.L., Day S. & Johnston I. (1993) Idiopathic pulmonary fibrosis and hepatitis C virus infection. *American Review of Respiratory Disease* (in press).

Kanai K., Kako M. & Okamoto H. (1992) HCV genotypes in chronic hepatitis C and response to interferon. *Lancet* **339,** 1543.

Kao J-H., Chen P-J., Yang P-M., Lai M-Y., Sheu J-C., Wang T-H. & Chen D-S. (1992) Intrafamilial transmission of hepatitis C virus: the important role of infections between spouses. *J. Inf. Dis.* **166,** 900–903.

Klein R.S., Freeman K., Taylor P.E. & Stevens C.E. (1991) Occupational risk for hepatitis C virus infection among New York City dentists. *Lancet* **338,** 1539–1542.

Kleter G.E.M., Brouwer J.T., Heijtink R.A., Schalm S.W. & Quint W.G.V. (1993) Detection of hepatitis C virus RNA in patients with chronic hepatitis C virus infections during and after therapy with alpha interferon. *Antimicrob. Agents Chemother.* **37,** 595–597.

Kuo G., Choo Q.L., Alter H.J., Gitnick G.L., Redeker A.G., Purcell R.H., Miyamura T., Dienstag J.L., Alter M.J., Stevens C.E., Tegtmeier F., Bonino F., Columbo M., Lee W-S., Kuo C., Berger K., Schuster J.R., Overby L.R., Bradley D.W. & Houghton M. (1989) An assay for circulating antibodies to a major etiologic virus of human non-A. non-B hepatitis. *Science* **244,** 362–364.

Lenzi M., Johnson P.J., McFarlane I.G., Ballardini G., Smith H.M., McFarlane B.M., Bridger C., Vergani D., Bianchi F.B. & Williams R. (1991) Antibodies to hepatitis C virus in autoimmune liver disease: evidence for geographical heterogeneity. *Lancet* **338,** 277–80.

McFarlane I.G., Smith H.M., Johnson P.J., Bray G.P., Vergani D. & Williams R. (1990) Hepatitis C virus antibodies in chronic active hepatitis: pathogenetic factor or false-positive result? *Lancet* **335,** 754–757.

McOmish F., Chan S-W., Dow B.C., Gillon J., Frame W.D., Crawford R.J., Yap P-L., Follett E.A.C. & Simmonds P. (1993) Detection of three types of hepatitis C virus in blood donors: investigation of type-specific differences in serologic reactivity and rate of alanine aminotransferase abnormalities. *Transfusion* **33,** 7–13.

Miller R.H. & Purcell R.H. (1990) Hepatitis C shares amino acid sequence similarity with pestiviruses and flaviviruses as well as members of two plant virus supergroups. *Proc Natl Acad Sci, USA* **87,** 2057–2061.

Mishiro S., Hoshi Y., Takeda K., Yoshikawa A., Gotanda T., Takahashi K., Akahane Y., Yoshizawa H., Okamoto H., Tsuda F., Peterson D.A. & Muchmore E. (1990) Non-A, non-B hepatitis specific antibodies directed at host-derived epitope: implication for an autoimmune process. *Lancet* **336,** 1400–1403.

Mitsui T., Iwano K., Masuko K., Yamazaki C., Okamoto H., Tsuda F., Tanaka T. & Mishiro S. (1992) Hepatitis C virus infection in medical personnel after needlestick accident. *Hepatology* **16,** 1109–1114.

Okamoto H., Sugiyami Y., Okada S., Kurai K., Akahane Y., Sugai Y., Tanaka T., Sato K., Tsuda F., Miyakawa Y. & Mayumi M. (1992) Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. *J. Gen. Virol* **73,** 673–679.

Omata M., Yokosuka O., Takano S., Kato N., Hosoda K., Imazeki F., Tada M., Ito Y. & Ohto M. (1991) Resolution of acute hepatitis C after therapy with natural beta interferon. *Lancet* **338,** 914–915.

Pascual M., Perrin L., Giostra E. & Schifferli J.A. (1990) Hepatitis C virus in patients with cryoglobulinaemia type II. *J. Infect. Dis.* **162,** 569–570.

Peano G.M., Fenoglio L.M., Menardi G., Balbo R., Marenchino D. & Fenoglio S. (1993) Heterosexual transmission of hepatitis C virus in family groups without risk factors. *Br. Med. J.* **305,** 1473–1474.

Pereira B.J.G., Milford E.L., Kirkman R.L., Quan S., Sayre K.R., Johnson P.J., Wilber J.C. & Levey A.S. (1992) Prevalence of hepatitis C virus RNA in organ donors positive for hepatitis C antibody and in the recipients of their organs. *New Engl. J. Med.* **327,** 910–915.

Reichard O., Andersson J., Schvarcz R. & Weiland O. (1991) Ribavirin treatment for chronic hepatitis C. *Lancet* **337,** 1058–1061.

Reinus J.F., Leikin E.L., Alter H.J., Cheung L., Shindo M., Jett B., Piazza S. & Shih J.W-K. (1992) Failure to detect vertical transmission of hepatitis C. *Ann. Int. Med.* **117,** 881–886.

Saeed A.A., Al-Admawi A.M., Al-Rasheed A., Fairclough D., Bacchus R., Ring C. & Garson J. (1991) Hepatitis C virus infection in Egyptian volunteer blood donors in Riyadh. *Lancet* **338,** 459–460.

SEEFF L.B., BUSKELL-BALES Z., WRIGHT E.C., DURAKO S.J., ALTER H.J., IBER F.L., HOLLINGER F.B., GITNICK G., KNODELL R.G., PERRILLO R.P., STEVENS C.E., HOLLINGSWORTH C.G. & THE NATIONAL HEART, LUNG AND BLOOD INSTITUTE STUDY GROUP (1992) Long-term mortality after transfusion-associated non-A. non-B hepatitis. *New Engl. J. Med.* **327,** 1906–1911.

SIMMONDS P., MCOMISH F., YAP P.L., CHAN S-W., LIN C.K., DUSHEIKO G., SAEED A.A. & HOLMES E.C. (1993a) Sequence variability in the 5′ non-coding region of hepatitis C virus: identification of a new virus type and restrictions on sequence diversity. *J. Gen. Virol.* **74,** 661–668.

SIMMONDS P., ROSE K., GRAHAM S., CHAN S-W., MCOMISH F., DOW B.C., FOLLETT E.A.C., YAP P.L. & MARSDEN H. (1993b) Mapping of serotype-specific, immunodominant epitopes in the NS-4 region of hepatitis C virus: use of type-specific peptides to serologically differentiate infection with HCV types 1, 2 and 3. *J. Clin. Micro.* (in press).

SIMONETTI R.G., CAMMA C., FIORELLO F., COTTONE M., RAPICETTA M., MARINO L., FIORENTINO G., CRAXI A., CICCAGLIONE A., GIUSEPPETTI R., STROFFOLINI T. & PAGLIARO L. (1992) Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. *Ann. Int. Med.* **116,** 97–102.

SUGITANI M., INCHAUSPE G., SHINDO M. & PRINCE A.M. (1992) Sensitivity of serological assays to identify blood donors with hepatitis C viraemia. *Lancet* **339,** 1018–1019.

THALER M.M., PARK C-K., LANDERS D.V., WARA D.W., HOUGHTON M., VEEREMAN-WAUTERS G., SWEET R.L. & HAN J.H. (1991) Vertical transmission of hepatitis C virus. *Lancet* **338,** 17–18.

TREMOLADA F., BENVEGNU L., CASARIN C., PONTISSO P., TAGGER A. & ALBERTI A. (1990) Antibody to hepatitis C virus in hepatocellular carcinoma. *Lancet* **335,** 300–301.

UEDA T., OHTA K., SUZUKI N., YAMAGUCHI M., HIRAI K., HORIUCHI T., WATANABE J., MIYAMOTO T. & ITO K. (1992) Idiopathic pulmonary fibrosis and high prevalence of serum antibodies to hepatitis C virus. *Am. Rev. Respir. Dis.* **146,** 266–268.

WANG J-T., WANG T-H., SHEU J-C., LIN J-T. & CHEN D-S. (1992) Hepatitis C virus RNA in saliva of patients with posttransfusion hepatitis and low efficiency of transmission among spouses. *J. Med. Virol.* **36,** 28–31.

WEJSTAL R., WIDELL A., MANSSON A-S., HERMODSSON S. & NORKRANS G. (1992) Mother-to-infant transmission of hepatitis C virus. *Ann. Int. Med.* **117,** 887–890.

ZAAIJER H.L., CUYPERS H.T.M., REESINK H.W., WINKEL I.N., GERKEN G., LELIE P.N. (1993) Reliability of polymerase chain reaction for detection of hepatitis C virus. *Lancet* **341,** 722–724.