negative and there is a strong suspicion of AICH, anti-LKM antibodies or positive tests for HCV may suggest type 2 AICH.

In the absence of other positive results metabolic disorders must be excluded. Serum copper, urinary copper excretion and plasma caeruloplasmin will help to exclude Wilson's disease. Serum ferritin and transferrin and α_1-antitrypsin levels should be determined to exclude iron storage diseases and α_1-antitrypsin deficiency.

In most instances liver biopsy is important. An adequate core is essential to ensure that sufficient representative tissue is present for a valid assessment to be made. Biopsies should be interpreted by an experienced pathologist to whom all the patient's clinical and biochemical findings should be available. Immunohistochemical techniques can be important in confirming a diagnosis; where such expertise is absent, the specimen should be sent to a centre of excellence for review. The block itself should accompany the slides so that further preparation is possible.

Where all diagnostic approaches fail to define an obvious cause, a 'non-diagnosis' of cryptogenic CH may have to be made. It is, however, important to remember that HCV infection, HBV infection and AICH will in some cases fail to yield definitively positive results. Here a trial of steroids should be undertaken, under strict supervision since viral CH may advance rapidly on steroid therapy.

In all but the most straightforward cases patients may benefit from review by a specialist centre interested in liver disease. It is our experience that many patients referred to the Liver Clinic of the University of Cape Town for consideration of a transplant for apparently 'end-stage' liver disease have on review turned out to have diseases amenable to medical therapy; in many instances this has made surgery unnecessary.

REFERENCES

1. DeGroote J, Desmet J, Gedigk P, Korb G, Popper H, Poulsen H, *et al.* A classification of chronic hepatitis. *Lancet* 1968; **2:** 626-628.
2. Geall MG, Schoenfield JJ, Summerskill WHJ. Classification and treatment of chronic active liver disease. *Gastroenterology* 1968; **55:** 724-729.
3. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol* 1991; **13:** 372-374.
4. Maddrey WC. Chronic hepatitis. *Dis Mon* 1993; **34**(2): 53-126.
5. Brown JL, Carman WF, Thomas HC. The hepatitis B virus. *Baillieres Clin Gastroenterol* 1990; **4:** 721-747.
6. Lavine JE, Bull FG, Millward-Sadler GH, Arthur MJP. Acute viral hepatitis. In: Millward-Sadler GH, Wright R, Arthur MJP, eds. *Wright's Liver and Biliary Disease*. London: WB Saunders, 1992: 679-786.
7. Jacyna MR, Millward-Sadler GH, Thomas HC. Chronic hepatitis. In: Millward-Sadler GH, Wright R, Arthur MJP, eds. *Wright's Liver and Biliary Disease*. London: WB Saunders, 1992: 787-820.
8. Deodhar KP, Tapp E, Scheuer PJ. Orcein staining of hepatitis B antigen in paraffin sections of liver biopsies. *J Clin Pathol* 1975; **28:** 66-70.
9. Hadziyannis SJ, Gerber MA, Vissoulis C, Popper H. Cytoplasmic hepatitis B antigen in 'ground-glass' hepatocytes of carriers. *Arch Pathol* 1973; **96:** 327-330.
10. Chazouilleres O, Mamish D, Kim M, Carey K, Ferrell L, Roberts JP, *et al.* 'Occult' hepatitis B virus as source of infection in liver transplant recipients. *Lancet* 1994; **343:** 142-146.
11. Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, *et al.* The natural history of community-acquired hepatitis C in the United States. *N Engl J Med* 1992; **327:** 1899-1905.
12. Jeffers LJ, Hasan F, De Medina M, Reddy R, Parker T, Silva M, *et al.* Prevalence of antibodies to hepatitis C virus among patients with cryptogenic chronic hepatitis and cirrhosis. *Hepatology* 1992; **15:** 187-190.
13. Trépo C, Zoulim F, Alonso C, Petit M-A, Pichoud C, Vitvitski L. Diagnostic markers of viral hepatitis B and C. *Gut* 1993; **34**(2): suppl, S20-S25.
14. Mattsson L. Chronic NANB hepatitis. *Scand J Infect Dis* 1989; suppl 59, 7-55.
15. Waldenstrom J. Leber blutproteine und Nahrungeiweiss. *Dtsch Z Verdau Stoffwechselkr* 1950; **2:** 113.
16. Kenny RP, Czaja AJ, Ludwig J, Dickson ER. Frequency and significance of antimitochondrial antibodies in severe chronic active hepatitis. *Dig Dis Sci* 1986; **31:** 705-711.
17. Todros J, Touscoz G, D'Urso ND, Durazzo M, Albano E, Poli G, *et al.* Hepatitis C virus-related chronic liver disease with autoantibodies to liver-kidney microsomes (LKM). *J Hepatol* 1991; **13:** 128-131.
18. Magrin S, Craxi A, Fiorentino G, Fabiano C, Provenzano G, Pinzello GB, *et al.* Is autoimmune chronic active hepatitis a HCV-related disease? *J Hepatol* 1991; **13:** 56-60.
19. Lenzi M, Johnson PJ, McFarlane IG, Ballardini G, Smith HM, McFarlane BM, *et al.* Antibodies to hepatitis C virus in autoimmune liver disease: evidence for geographical heterogeneity. *Lancet* 1991; **338:** 277-280.
20. Williamson JMS, Chalmers DM, Clayden AD, Dixon MF, Ruddell WS, Losowsky MS. Primary biliary cirrhosis and chronic active hepatitis: An examination of clinical, biochemical, and histopathological features in differential diagnosis. *J Clin Pathol* 1985; **38:** 1007-1012.
21. Seeff LB. Drug-induced chronic liver disease with emphasis on chronic active hepatitis. *Semin Liver Dis* 1981; **1:** 104-115.
22. Maddrey WC. Drug-induced chronic liver disease. *Gastroenterology* 1977; **72:** 1348-1353.
23. Seggie J, Saunders SJ, Kirsch RE, Campbell JA, Gitlin N, Clain D, *et al.* Patterns of hepatic injury induced by methyldopa. *S Afr Med J* 1979; **55:** 75-83.
24. Moses A, Zahger D, Amir G. Cholestatic liver injury after prolonged exposure to methyldopa. *Digestion* 1989; **42:** 57-60.
25. Stanley P, Mijich A. Methyldopa: an often overlooked cause of fever and transient hepatocellular dysfunction. *Med J Aust* 1986; **144:** 603-605.
26. Breland BD, Hicks GS Jr. Hepatitis and hemolytic anaemia associated with methyldopa therapy. *Drug Intell Clin Pharmacol* 1982; **16:** 489-492.
27. Reynolds TB, Peters RL, Yamada S. Chronic active and lupoid hepatitis caused by a laxative, oxyphenisatin. *N Engl J Med* 1971; **285:** 813-820.
28. Sharp JR, Ishak KG, Zimmerman HJ. Chronic active hepatitis and severe hepatic necrosis associated with nitrofurantoin. *Ann Intern Med* 1980; **92:** 14-19.
29. Maddrey WC. Alcoholic hepatitis: pathogenesis and approaches to treatment. *Scand J Gastroenterol Suppl* 1990; **175:** 118-130.
30. Brillanti S, Masci C, Siringo S, *et al.* Serological and histological aspects of hepatitis C virus infection in alcoholic patients. *J Hepatol* 1991; **13:** 347-350.
31. Schaffner F, Thaler H. Nonalcoholic fatty liver disease. *Prog Liver Dis* 1986; **8:** 283-298.
32. Powell EE, Cooksley GE, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990; **11:** 74-80.
33. Scheinberg IH, Sternlieb I. *Wilson's disease* (Major Problems in Internal Medicine, vol. 23). Philadelphia: WB Saunders, 1984.
34. LaRusso NF, Summerskill WHJ, McCall JT. Abnormalities of chemical tests for copper metabolism in chronic active liver disease: differentiation from Wilson's disease. Gastroenterology 1976; **70:** 653-655.
35. Sternlieb I, Scheinberg IH. Wilson's disease. In: Millward-Sadler GH, Wright R, Arthur MJP, eds. *Wright's Liver and Biliary Disease*. London: WB Saunders, 1992: 965-975.
36. Fleming CR, Dickson ER, Wahner HW, Hollenhorst RW, McCall JT. Pigmented corneal rings in non-Wilsonian liver disease. *Ann Intern Med* 1977; **86:** 285-288.
37. Kaplinsky C, Sternlieb I, Javitt N, Rotem Y. Familial cholestatic cirrhosis associated with Kayser-Fleischer rings. *Pediatrics* 1980; **65:** 782-788.
38. Sharp HL. Alpha-1 antitrypsin: an ignored protein in understanding liver disease. *Semin Liver Dis* 1982; **2:** 314-328.
39. Triger DR, Carlson J, Millward-Sadler GH. Alpha-1-antitrypsin deficiency and liver disease. In: Millward-Sadler GH, Wright R, Arthur MJP, eds. *Wright's Liver and Biliary Disease*. London: WB Saunders, 1992: 1170-1188.
40. Summers KM, Halliday JW, Powell LW. Identification of homozygous haemochromatosis subjects by measurement of hepatic iron index. *Hepatology* 1990; **12:** 20-25.

Management of chronic hepatitis B and C

G. M. Dusheiko

Chronic viral hepatitis caused by hepatitis B, C or D may lead to cirrhosis, hepatocellular failure and hepatocellular carcinoma. The morbidity of these diseases has necessitated a prolonged search for effective therapy. Although many antiviral compounds have been evaluated for the treatment of chronic viral hepatitis, few have achieved clinical applicability. Alpha-interferon has been widely studied, and remains the mainstay of treatment. A number of other cytokines, including thymosin, are being evaluated. Nucleoside analogues, alone or in combination with alpha-interferon, may prove useful adjuncts to the treatment of chronic hepatitis B and C.

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Antiviral treatment of hepatitis B

Hepatitis B is caused by the hepatitis B virus (HBV), a large (42 nm) enveloped DNA virus that infects the liver, causing hepatocellular necrosis and inflammation. The infection can be either acute or chronic, and can range in severity from an asymptomatic infection that resolves completely, to a severe, symptomatic infection with progressive and even fatal illness. Some patients with chronic hepatitis B have a remission in disease, marked by disappearance of HBV DNA and HBeAg from serum with improvement in serum aminotransferase activity despite persistence of HBsAg. Measurement of serum HBV DNA in patients with chronic hepatitis B by polymerase chain reaction (PCR) demonstrates that the loss of HBeAg is associated with a decreased rather than complete clearance of HBV DNA in the serum.[1] Other patients lose HBeAg or are anti-HBe positive, yet have active liver disease and HBV DNA detectable in serum by dot or blot hybridisation techniques. These patients have been shown to be infected with an HBV mutant, with a nucleotide substitution in the pre-core region of the genome.[2,3]

Antiviral therapy for chronic hepatitis B

Interventional treatment of chronic hepatitis B is targeted at patients with active disease and viral replication, preferably at a stage before signs and symptoms of cirrhosis or significant injury have occurred. Eradication of the infection is possible in only a minority of patients. Permanent loss of HBV DNA and HBeAg is considered a response to antiviral treatment, as this result is associated with an improvement in necro-inflammatory damage and reduced infectivity. It is possible, but unproven, that the accompanying reduction in histological chronic active hepatitis lessens the risk of cirrhosis and hepatocellular carcinoma. HBV DNA may still be detectable in serum and liver after loss of HBeAg and HBsAg induced by antiviral therapy, but this is often not associated with progressive disease.[4]

Alpha-interferon

The interferons are a family of related proteins. There are four main groups: alpha, beta, omega and gamma. There are at least 24 interferon-alpha genes but only single beta and gamma genes. Interferon-alpha-1 (alpha and gamma) genes code for 165-166 amino acids. Type II (gamma) interferon genes code for 174 amino acids. Interferon-alpha and gamma are induced in most nucleated cells in response to virus infection or to double-stranded RNA (ds RNA). They act via a common surface receptor.

Recombinant and lymphoblastoid interferons are available for therapeutic use. Therapy for hepatitis B has now become possible with the demonstration that alpha interferons inhibit HBV replication and that prolonged therapy can lead to a remission in disease, although the mechanism of action of interferons in chronic hepatitis B infection is not known. Unfortunately, interferon therapy of chronic hepatitis is effective in fewer than 50% of cases of chronic hepatitis B, is relatively expensive, requires administration by injection, is often confusing in its effects, and is not without side-effects. Nonetheless, recombinant interferon has been licensed for treatment of chronic hepatitis B in many countries.

Antiviral mechanisms of interferons

Interferons interfere with several stages of the viral life cycle, although the actual mechanism varies with different viruses. They induce several enzymes and proteins, the best characterised of which are the 2'5'-oligo-adenylate synthetases (2'5'-A synthetases), ds RNA-dependent protein kinases and the Mx system.

Other antiviral mechanisms include the activation of expression of the class I major histocompatibility antigen (MHC) genes by all interferons, and those of the class II by gamma interferon, to increase and enhance the expression of MHC at the cell surface, and thereby amplify viral antigen recognition and display. Interferons also modify the cellular and humoral immune response; expression and activation of natural killer cells and T lymphocytes are affected. Early and late events in the viral life cycle, such as uncoating of viruses or integration of proviral DNA, are modified by interferons.

Viruses may decrease interferon production from infected cells, and certain viruses may have evolved mechanisms of inhibition of interferon production.[5] It has indeed been suggested that a rationale for interferon treatment of chronic hepatitis B is a relative defect in adult carriers in the production or action of interferon, or an inhibition by hepatitis B of the activation of the interferon system.[6,7] In particular, interferon is not detectable in the serum of patients chronically infected with HBV.

Therapeutic use of alpha-interferon in chronic hepatitis B

Alpha, beta and gamma interferons were recombinantly cloned and became available for therapeutic use after 1981. Three preparations of alpha-interferon are currently available, two of which are recombinant (Roferon A, alpha-2a, Hoffmann La Roche) (Intron A, alpha-2b, Schering), and one of which is prepared from a lymphoblastoid cell line (Wellferon, alpha n1, Burroughs Wellcome). Alpha-2a and 2b are recombinant preparations produced in *Escherichia coli*. Alpha-2a has a lysine molecule at position 23 instead of the arginine present in alpha-2b. Alpha-n1 is a natural mixture of interferon-alpha produced by human lymphoblastoid cells after viral stimulation. Early pilot studies pointed out the inhibitory effect of recombinant alpha-2a interferon upon HBV replication and the therapeutic potential of this drug.[8] Later, in controlled dose-finding studies, predictors of response could be tentatively defined, dose ranges narrowed and therapeutic strategies outlined.[9] Approximately 35 - 45% of patients treated with 5 - 10 MU three times weekly for 6 months become negative for HBeAg.

Response rates in most of the early studies were higher in carriers with higher baseline serum aminotransferase levels

than in those without elevated aminotransferase levels. The subclinical exacerbation of the hepatitis frequently seen in responders suggests that interferon acts by augmenting the immune response to HBV, perhaps triggered by the inhibition of viral replication as well as the effects of interferon on cytotoxic T cells.

Based on the data obtained from these studies, indications for treatment and definitions of response can be formulated.[10] The immediate goal of therapy is to eradicate viral replication and ameliorate the underlying liver disease. Recommendations for therapy should be cautious, because therapy is effective in only 30 - 50% of patients, the optimal dose and duration of therapy remain unclear, and side-effects can be problematic. Alpha-interferon therapy is, however, indicated in patients with typical chronic hepatitis B who have HBsAg in serum with HBeAg and/or HBV DNA and raised aminotransferase activity (at least twice the upper limit of normal). Patients with HBsAg without HBV DNA in serum should not be treated, as alpha-interferon has no effect on HBsAg alone. A response to alpha-interferon is defined by a loss of HBV DNA and HBeAg from serum, fall of serum aminotransferase activities into the normal range and subsequent improvement in liver histology. Measurement of HBV DNA can be quantitated, but is not standardised.[11] HBeAg may not become undetectable for some months after HBV DNA is negative. Although residual HBV DNA can be detected by PCR in serum, the disease appears to be ameliorated.[4] Approximately 20% of patients who respond to treatment with clearance of HBeAg will also clear HBsAg within a year of therapy, and up to 65% may later clear HBsAG after 6 years of follow-up.[12]

Response is generally associated with histological reduction in inflammation. Although parameters that predict responsive patients are difficult to characterise fully, the following may respond more favourably: women, those carriers with elevated pre-treatment serum aminotransferase levels, those with chronic active hepatitis, those with lower serum HBV DNA, those negative for anti-HIV, those with a history of hepatitis, whites with more recent onset of disease, with IgM anti-HBc, increased expression of cyto-adhesion molecules and activation of the interferon systems as measured by 2'5'-oligo-adenylate synthetase.[13,14] HIV-positive patients tend to have higher levels of HBV replication[15] and are less responsive. Although these factors provide some predictive information, none of these criteria is absolute and individual carriers, e.g. ethnic Chinese with active disease or those patients with anti-HIV but normal CD4 lymphocyte counts, may respond, making the prediction of treatment outcome somewhat difficult.

The appropriate dose of interferon is not yet established, but 5 - 10 MU three times weekly, or 4,5 - 5 MU daily given subcutaneously or intramuscularly for 4 months is currently prescribed.

The major early side-effects of interferon include an influenza-like syndrome, chills, fever, malaise, muscle aches and rigors. Later side-effects include malaise, muscle aches, headaches, poor appetite, weight loss, increased need for sleep, psychological effects, (irritability, anxiety, depression) hair loss, thrombocytopenia and leukopenia. Unusual or severe side-effects include seizures, acute psychosis, bacterial infections, auto-immune reactions, thyroid disease, proteinuria, myocardiopathy, skin rashes and interferon antibodies. Patients should be monitored at 1 - 4-weekly intervals during treatment and blood counts and serum aminotransferase activity should be measured at these intervals. HBV DNA and HBeAg should be measured monthly to monitor the progress of treatment. Thyroid functions should be measured before, during and at the end of therapy.[16-19] There are recent reports of interstitial lung disease in patients treated with higher doses of alpha-interferon.

The role of interferon antibodies in reducing response is controversial, but neutralising of antibodies may be one variable which affects response to interferon. A higher proportion of patients with chronic hepatitis (20%) treated with alpha-2a develop neutralising antibody (20% v. 6,9% v. 1,2% among patients treated with alpha-n1). Thus interferon-alpha-2a may be more immunogenic.[20,21] In some patients loss of efficacy or 'breakthrough' may correlate with antibodies, and a neutralising effect may be responsible in some patients who have a breakthrough after an initial response, and in whom response occurs after treatment with an alternative interferon. However, it is still difficult to draw firm conclusions about the clinical significance of interferon antibodies. The patients treated are often heterogeneous, other factors affect the response and patients may respond despite developing antibodies. There are also different assays for measuring antibodies including immuno-assay, immunoradiometric assays, and antiviral neutralisation bio-assays.

Patients who do not respond to therapy with alpha-interferon represent a difficult management problem. Lower doses given for a longer period are currently being studied. The prescription of alpha-interferon is also limited by its relatively high cost, but an economic evaluation of the relative costs of alpha-interferon and chronic hepatitis B suggests that some benefit can be obtained.[22]

Treatment of special groups of patients with hepatitis B

Patients with anti-HBe-positive chronic hepatitis

Patients who are anti-HBe positive but who have HBcAg in hepatocytes and histological chronic active hepatitis due to infection with HBV pre-core mutants affecting the expression of HBeAg may have severe disease. In parts of Europe and elsewhere, disease caused by this variant of hepatitis B is more common than disease caused by the 'wild type' of HBeAg-positive chronic hepatitis. Approximately 25% of patients have long-term responses.[23] In a trial of interferon-alpha-2a therapy with 9 MU given intramuscularly three times weekly for 16 weeks, 33% of anti-HBe-positive carriers lost HBcAg, compared with none of the controls. In a second study of lymphoblastoid interferon (5 MU/m^2 for 6 months) in 30 patients, at 18 months, 53% of the treated patients had responded v. 17% of the controls.[24] Histological improvement was seen in 53% and HBcAg disappeared in 66%, compared with 33% of untreated patients. Longer-term evaluation has indicated that patients have tended to relapse with time. Thus although response rates are high, many patients relapse when treatment is stopped.

Table I. Other antiviral therapies investigated for the treatment of chronic hepatitis B

Gamma-interferon
Acyclovir (acycloguanosine, ACV)
Foscarnet (trisodium phosphonoformate)
Nucleoside analogues
2'3'-dideoxyguansine (ddG), 2',3' -dideoxyinosine (ddI), 3'-azido-2' dideoxythymidine (AZT)
Adenine arabinoside
Ara-AMP conjugated with lactosaminated albumin
Levamisole
Phyllanthus amarus
Isoprinosine
Interleukin-2
Thymosin
Tumour necrosis factor

Combination therapies
Pulsed corticosteroid treatment and interferon
Gamma- and alpha-interferon
Gamma- and beta-interferon
Acyclovir and interferon-alpha
Steroid withdrawal and acyclovir

New experimental nucleoside analogues
2' -fluorinated pyrimidine arabinosyl furanosides
2,6, diaminopurine 2',3', dideoxyriboside
2',3' -dideoxy-3' -fluorothymidine (FddThd), 2',3'-dideoxy-3'-fluoro-5-methylcytidine (FddMeCyt), 2', 3'-dideoxy-3'-fluoro-5-ethylcytidine (FddEtCyt),
The 2'-fluorinated arabinosyl-pyrimidine nucleosides: FIAC, FMAU
Famciclovir
2',3'-dideoxy-3'-thiacytidine ((±)-SddC) (Lamuvidine)
(2'-deoxy-3'-thia-5-fluorocytosine (FTC))
Purine nucleoside analogues (cyclobut A and cyclobut G) with cyclobutane rings
(–) and (+) enantiomers of cis-5-fluoro-1- (2-(hydroxymethyl)-1, 3-oxathiolan-5-yl) cytosine
erythromycin A-9-methyloxime (EMO) and other oxime derivatives of erythromycin A

Other modalities
Antisense oligodeoxyribonucleotides
Synthetic oligodeoxynucleotide coding for ribozyme motifs

Treatment of children

Asian children, or other children who are infected perinatally, have mild disease activity and respond poorly to interferon treatment.[25] They appear to be profoundly tolerant of HBV, perhaps as a result of developing tolerance to HBeAg in the neonatal thymus, and thereby impairing the clonal T cell response to HBV.[26] In contrast, children with active disease and high serum aminotransferase levels respond to interferon therapy similarly to adults and usually accept treatment well.[27,28] The appropriate dose of interferon in children is 5 MU/m^2 subcutaneously three times weekly for 4 months.

Oriental patients

Prospective studies have shown that Asians have a low rate of response to alpha-interferon and frequently relapse.[25,29] For these reasons, alpha-interferon therapy should be limited to those Oriental patients with active disease and elevations in serum aminotransferase levels. Furthermore, these patients should be treated only after explanation of the low possibility of response. Patients with active disease may respond to corticosteroid withdrawal and interferon; in a preliminary report, up to 50% of patients with elevated ALT cleared HBeAg.[30]

Patients with advanced cirrhosis

Patients with cirrhosis and ascites, encephalopathy or persistent jaundice from chronic hepatitis B have a dire prognosis without treatment, but are prone to developing multiple, serious side-effects from higher doses (2 - 10 MU) of alpha-interferon, particularly bone marrow suppression, neutropenia and an increased incidence of serious bacterial infections. Low doses of interferon 1- 3 MU three times a week could be considered in these patients.[31] This treatment should be administered cautiously, and only by physicians with experience of interferon, who can monitor the patients weekly, and where liver transplantation is available. Therapeutic trials of low doses of alpha-interferon in such patients are in progress.

Immunosuppressed patients

Chronic hepatitis B is common among patients on renal dialysis, patients with malignancies receiving chemotherapy and patients who have had kidney, heart or liver transplants. Anecdotal experience suggests that interferon has little effect in patients with major immune deficiencies.[29] Retrospective analyses have suggested that patients with anti-HIV do not respond to interferon as well as non-HIV-infected patients. However, good responses may occur in some patients with anti-HIV but normal peripheral CD4 T lymphocyte counts, so that perhaps anti-HIV alone should not exclude a patient from therapy.

Patients with extrahepatic disease

Glomerulonephritis caused by chronic HBV has been treated with recombinant interferon-alpha-2b at a dose of 5 million units subcutaneously daily.[33] In patients in whom HBeAg disappeared and aminotransferase levels fell within the normal range, urine protein excretion also decreased and glomerulonephritis resolved.

Other antiviral agents for chronic hepatitis B

Other antiviral therapies for chronic hepatitis B have made use of a variety of agents. These include the following.

Currently used nucleoside analogues

Antiretroviral therapies used in HIV infection have been tested in chronic hepatitis B. Both these viruses contain a reverse transcriptase. Initial experiments in hepatoblastoma cells transfected with hepatitis B (hepG2) suggested that 2'3'-dideoxyguansine (ddG), 2'3'-dideoxyinosine (ddI) and 3'-azido-2'dideoxythymidine (AZT) diminished HBV replication by suppressing reverse transcription in the replicative cycle.[33] However, their usefulness in patients is limited.

Adenine arabinoside is an analogue of adenine, and a potent inhibitor of HBV DNA polymerase. Adenine arabinoside 5'-monophosphate (ara-AMP), a water-soluble congener, can be administered intramuscularly. Suppression of HBV DNA has been demonstrated and clearance of HBeAg in 33% of patients were achieved in recent controlled trials. Usually 8 weeks of treatment are required to effect loss of HBeAg and HBV DNA, but in many cases, serious neurotoxicity is seen after 4 weeks. Although the drug is a potent inhibitor of hepatitis B virus, its future as single-agent therapy for chronic hepatitis B is uncertain. Antiviral therapy with ara-AMP may be effective in suppressing the disease activity and improving survival in patients with polyarteritis.[34]

Ara-AMP conjugated with lactosaminated albumin, a galactosyl-terminating neoglycoprotein which selectively penetrates into hepatocytes by receptor-mediated endocytosis, inhibited virus replication in patients with HBV infection and woodchucks with chronic WHV infection. Free and conjugated ara-AMP were active at doses of 10 mg/kg and 0,75 mg/kg respectively.[35] This strategy may reduce the dose related side-effects; however, the compound is not generally available. Hepatocyte vacuoles caused by swelling of secondary lysosomes have been reported in cases where high doses of the conjugate were administered to experimental animals.[36]

Levamisole, an anti-helminthic, has been claimed to inhibit HBV replication in up to 60% of patients.[37] However, it has not yet been established whether the initial good results have proved reproducible. A combined trial of lymphoblastoid interferon (5 MU/m^2 plus levamisole (150 mg three times per week)) revealed a higher response rate (38%) in patients treated with interferon alone compared with those treated with the combination (10%). Thus levamisole has no added benefit when combined with alpha-interferon.[38]

Thymosin is an immune stimulant known to enhance suppressor T-cell activity and *in vitro* B-cell synthesis of IgG. Peptide preparations of thymosin have been evaluated in small controlled trials, and HBV DNA has been noted to become negative in some of these patients, as well as in chimpanzees with chronic hepatitis B.[39] It is given parenterally. This interesting agent's possible therapeutic role is now being evaluated in a larger controlled trial. The drug has few side-effects, and may prove to be an alternative to alpha-interferon.

Combination therapies

Pulsed corticosteroid treatment and interferon

A multi-centre trial comparing the efficacy of prednisolone withdrawal plus interferon (alpha-2b) to interferon alone (5 MU daily for 16 weeks) has not shown any added benefit from corticosteroids plus interferon: 38% of patients in both groups lost HBeAg, and 12% and 10% respectively lost HBsAg.[40] However, the response rates with combined treatment were better than interferon alone in those patients with pre-treatment serum ALT less than 100 IU/l (44% v. 18%). These data could mean that 'unprimed' carriers, with inactive disease, who are poorly responsive, are rendered more responsive by prior activation with corticosteroid withdrawal. The investigators also showed that carriers with lower concentrations of HBV DNA (<100 pg/ml) were more likely to respond to treatment. This treatment regimen should not be used in patients with decompensated hepatitis B because of the risk of inducing severe hepatic necrosis.

New nucleoside analogues

The *in vitro* and *in vivo* antiviral effects of new purine and pyrimidine antiviral nucleoside analogues are currently a major research focus. This research has recently suffered a major setback with reports of lethal toxicity in patients with chronic hepatitis B treated with fialuridine. The preliminary findings point to a possible association of mitrochondrial toxicity with this and other nucleoside analogues.[41] The search therefore continues for safe drugs for the treatment of hepatitis B. There are several other promising and possibly safer drugs now being investigated, including drugs with less activity against mitochondrial gamma polymerase, for example 3-thiacytidine or famciclovir.[42] Their efficacy is presently unproven: chronic type B hepatitis disease will require relatively long courses of treatment, and many of these antiviral drugs may require activation of the immune response for eradication of HBV.

Antiviral treatment of HCV

Newer tests for diagnosis of hepatitis C include detection of antibodies to several recombinantly expressed HCV antigens, or peptides (c100-3, c22, c33, c200, NS5) and detection of HCV RNA in serum by polymerase chain reaction amplification. Several isolates of hepatitis C have been cloned. The sequence divergence of these isolates of hepatitis C have been cloned. The sequence divergence of these isolates indicates that there are several major genotypes (serotypes) of hepatitis C and component subtypes.[43,44] Geographical localisation of these types has been reported.

The natural history of the disease is uncertain. The virus has a disturbing propensity to cause chronic infection, and it is believed that 10 - 20% of patients with chronic hepatitis C infections will progress to cirrhosis within a decade. The risk of further progression is probably cumulative, but is influenced by other co-factors which include viral genotype, level of viraemia, other viral infection, immunosuppression, age of acquisition, alcohol and perhaps parasitic disease. The infection causes systemic disease and may be associated with a number of systemic complications, including a form of auto-immune hepatitis, cryoglobulinaemia, porphyria cutanea tarda, lymphocytic sialedinitis and membranous glomerulonephritis.

There are conflicting reports regarding the occurrence of hepatitis C antibodies in patients with auto-immune liver disease. Clearly the ELISA for anti-HCV is prone to false positive results in patients with high concentrations of immunoglobulins in serum.[45,46] However, in some countries, patients with type II auto-immune chronic active hepatitis appear to have a high frequency of genuine exposure to HCV, and antibodies to liver and kidney microsomal antibodies are present in patients.[47,48] These patients may

also have circulating antibodies to a pentadecapeptide (Gor), an epitope of normal hepatocytes; this phenomenon may represent an auto-immune response peculiar to type C hepatitis.[49] This association has some therapeutic implications — in some of these patients the disease may be aggravated by alpha-interferon and responsive to corticosteroids.

Therapy of chronic hepatitis C

Chronic hepatitis is a common consequence of community-acquired hepatitis C infection in patients who may not have suspected exposure to the virus. Prospective studies have suggested that 10 - 20% of patients with chronic NANB hepatitis develop cirrhosis within a 10-year period. Individuals with chronic hepatitis C with elevated ALT and histological evidence of chronic active hepatitis should therefore be considered for antiviral therapy. Asymptomatic patients detected through blood screening will require a supplement test to verify their HCV status, as the rate of false positive anti-HCV tests in low-risk donors is high. Ideally HCV RNA should be measured in all patients to confirm viraemia, but the test is not generally available for routine diagnosis. If the test is reproducibly positive, then levels of serum aminotransferases, bilirubin and alkaline phosphatase, and prothrombin time should be measured in referred blood donors. In patients whose lifestyle or geographic origin suggests that they are at risk of other forms of viral hepatitis, HBsAg and HIV infection also need to be considered. Because auto-immune hepatitis is treated differently, it is particularly advisable to exclude this diagnosis by measuring the titres of anti-smooth muscle and anti-liver kidney microsomal antibodies, even in those with a positive anti-HCV test, and to measure HCV RNA in anti-HCV-positive patients in whom interferon is contemplated.

Alpha-interferon therapy

Preliminary therapeutic trials of alpha-interferon indicated that a proportion of patients may respond to treatment with this agent. Larger, placebo-controlled studies have indicated that approximately 50% of patients will have normal serum aminotransferase activity after treatment courses of alpha-interferon of approximately 3 million units three times a week for 6 months.[50,51]

Serum HCV RNA may become undetectable in patients after 4 - 8 weeks of alpha-interferon treatment in patients who respond, but an undetectable HCV RNA at the end of treatment does not preclude relapse in patients.

However, after stopping treatment after 6 months, one-half of the responsive patients will promptly relapse. Serum aminotransferase levels usually increase in patients who are HCV RNA positive at the end of therapy, although in some cases the relapse may be delayed for several months.[52] Our studies at the Royal Free Hospital indicate that 20% of patients have a prolonged response to therapy and do not again develop elevated serum aminotransferase activity.[53] These patients also remain negative for HCV RNA.

There is most information about 3 MU three times a week given for 6 months. It is not yet clear whether this dose is optimal. Other regimens are being evaluated, and there is a suggestion that higher doses may be beneficial.[54] Initiating therapy with a somewhat higher dose of 15-18 million units per week, and prolonging therapy for a year may result in lower relapse rates; in a Japanese study of alpha-2b, it was concluded that 10 MIU of interferon administered 6 days a week for 2 weeks followed by three times a week for an additional 12 weeks produces the highest rate of both biochemical and virological responses to interferon therapy in patients with chronic HCV.[55] However, relapses still occur after higher doses, and the side-effects are greater at higher doses. The cost of 6 months of treatment is at least R7 500. Treatment should not be continued beyond 3 months in patients who do not have reduced levels of serum ALT. Responsive patients usually exhibit histological improvement, and may have a decrease in collagen III propeptide concentrations.[56]

Unfortunately responsiveness to alpha-interferon remains somewhat unpredictable. Factors which predict a greater likelihood of response are now being studied. Multivariate analyses of several pre-treatment parameters indicate that patients without cirrhosis are more responsive to interferon, and are more likely to have a sustained response. The influence of genotypes of hepatitis C is of considerable interest at present, as is the association between lower levels of viraemia and response. In Japanese studies on genotypes, it has become apparent that genotype II (Okamato or Simmonds 1b), the major genotype in Japan, is associated with a poor response to interferon therapy.[57,58] Similar differences in European patients have been reported. However, (Okamato) genotype III (Simmonds 2a) is sensitive to interferon in a high proportion of patients. Patients with diverse circulating quasispecies may be less responsive to therapy than those with a single major species. Improved responses have been observed in patients with lower levels of circulating HCV RNA.[59] Unfortunately, the issue remains complex: there is not yet a standardised system for quantitating concentrations of HCV RNA in serum. Also, these factors may be interdependent, as particular viral strains may replicate at higher efficiency than others. Genotyping and HCV RNA quantitation are not generally available to clinicians. However, when serotyping becomes available together with quantitation of HCV RNA, clinicians may be able to rationalise the use of interferon and the dose required.

When can treatment be considered successful? These criteria vary, but it is reasonable to infer that those patients with normal serum ALT a year after stopping interferon treatment, and negative for HCV RNA a year after stopping therapy, with histologically improved disease activity and perhaps a normal serum procollagen III peptide concentration, have had a good response. HCV antigens may be cleared from the liver with successful treatment.[60]

Some patients have a sustained response to treatment; a small proportion (approximately 10 - 20%) of these patients may be HCV RNA-positive, but appear to have a higher probability of later relapse off therapy. Others have a transient response, have normal serum ALT on treatment, and relapse soon after treatment is stopped. Other patients may have a partial response with improvement but not normalisation of the serum aminotransferases. Unfortunately a proportion of patients may have a good initial response to treatment, but then the ALT activity rises again despite continuing treatment; it is possible that some patients develop neutralising interferon antibodies. The timing of

antibody development may be a factor in explaining the course of responsive patients who do develop neutralising antibodies.[21] Continuing interferon with a second course of treatment with a different interferon may be useful in some patients. Only a small number of patients have been reported, however. The significance of response to a different interferon after unsuccessful treatment with a first interferon is less certain.

Other patients do not respond to treatment, and no improvement in serum ALT activity can be discerned. Some patients may actually worsen on treatment with interferon, and develop increased serum aminotransferases. A positive anti-HCV antibody in patients with auto-immune disease remains a pitfall in diagnosis, which has implications for treatment.[61] Such patients require confirmation by immunoblot assay, or HCV RNA, as they may optimally require corticosteroid therapy rather than alpha-interferon.[61] It is possible that such patients have an underlying auto-immune status associated with hepatitis C and exacerbated by interferon treatment. For such patients, and for patients who do not respond to treatment, ribavirin may be an alternative.

Immunosuppressed patients and patients with HIV may respond although long-term responsiveness is uncertain. This is of particular importance in liver transplant patients.

Ribavirin

Ribavirin is a synthetic guanosine nucleotide analogue, which possesses a broad spectrum of activity against both DNA and RNA viruses *in vitro* and *in vivo*.[64] Efficacy has been demonstrated in several viral diseases. The drug exerts its action after intracellular phosphorylation to mono-, di- and triphosphate nucleotides. The precise mode of action probably includes perturbation of intracellular nucleoside triphosphate pools, interference with the formation of the 5' cap structure of viral mRNA by competitive inhibition of both guanyltransferase- and methyltransferase-capping enzymes, direct inhibition of the viral mRNA polymerase complex, and possibly enhancement of macrophage inhibition of viral replication.

The pharmacokinetics of ribavirin have been studied. The bio-availability of oral formulations has been calculated at 19 - 65% (compared with intravenous administration). The distribution half-life is 1 - 3 hours, but the terminal half life is prolonged (27 - 52 hours), perhaps due to sequestration within red cells and other tissues. Ribavirin is concentrated 10 - 50-fold in red blood cells, and crosses the blood-brain barrier. Peak plasma levels range from 54 uM to 13 uM after single oral doses of 600 - 2 400 mg. The excretion of the drug is predominantly renal.

The major side-effects of the drug that have been reported include anaemia, a metallic taste, dry mouth, flatulence, dyspepsia, nausea, headaches, irritability, emotional lability, fatigue, insomnia, skin rashes and myalgia. Mild reversible anaemia is common. Modest increases in uric acid have been reported.

Studies of ribavirin in hepatitis C infection

In an open-label study in Sweden, in which ribavirin was prescribed to 10 patients with chronic hepatitis C (1 000 - 1 200 mg/day) for 12 weeks, median serum AST levels declined, but rose to pre-treatment levels upon completion.[63] A small study, using escalating doses of ribavirin (600 - 1 200 mg) showed a somewhat slower fall in serum aminotransferases, perhaps reflecting the lower starting dose of ribavirin.[64] There was a significant decrease in geometric mean titres of HCV RNA. At the Royal Free Hospital, we have treated 40 patients with chronic hepatitis C.[65] Thirty-eight per cent had normal ALT during treatment, but a further one-third had only a 50% decline in ALT. The remaining third were not responsive. The time course of normalisation of ribavirin is slower (mean time to normal — 5,5 months) in patients with biochemical improvement. Despite the significant improvement in serum ALT, we have not observed a marked decline in serum HCV concentrations. Further controlled trials to assess response and relapse rates are warranted, and a multicentre placebo-controlled trial of ribavirin for hepatitis C is now in progress. Studies of ribavirin and alpha-interferon used sequentially or together are also indicated, as preliminary reports indicate that the combination of treatment is associated with higher response rates than interferon alone. The multicentre placebo-controlled trial of ribavirin for hepatitis C is now in being analysed.

REFERENCES

1. Kaneko S, Miller RH, Di Bisceglie AM, Feinstone SM, Hoofnagle JH, Purcell RH. Detection of hepatitis B virus DNA in serum by polymerase chain reaction. *Gastroenterology* 1990; **99:** 799-804.
2. Carman WF, Jacyna MR, Hadziyannis S, *et al.* Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989; **2:**588-591.
3. Brunetto MR, Stemler M, Bonino F, *et al.* A new hepatitis B virus strain in patients with severe anti-HBe positive chronic hepatitis B. *J Hepatol* 1990; **10:** 258-261.
4. Marcellin P, Martinot-Peignoux M, Loriot MA, *et al.* Persistence of hepatitis B virus DNA demonstrated by polymerase chain reaction in serum and liver after loss of HBsAg induced by antiviral therapy. *Ann Intern Med* 1990; **112:** 227-228.
5. Schmilovitz-Weiss H, Levy M, Thompson N, Dusheiko G. Viral markers in the treatment of hepatitis B and C. *Gut* 1993; **34:** suppl, S26-S35.
6. Onji M, Lever AM, Saito I, Thomas HC. Defective response to interferons in cells transfected with the hepatitis B virus genome. *Hepatology* 1989; **9:**92-96.
7. Twu JS, Lee CH, Lin PM, Schloemer RH. Hepatitis B virus suppresses expression of human beta-interferon. *Proc Natl Acad Sci USA* 1988; **85:** 252-256.
8. Dusheiko G, Di Bisceglie A, Bowyer S, *et al.* Recombinant leukocyte interferon treatment of chronic hepatitis B. *Hepatology* 1985; **5:** 556-560.
9. Dusheiko GM, Paterson AC, Pitcher L, *et al.* Recombinant leucocyte interferon treatment of chronic hepatitis B. An analysis of two therapeutic trials. *J Hepatol* 1986; **3:** suppl 2, S199-S207.
10. Hoofnagle JH. Chronic hepatitis B. *N Engl J Med* 1990; **323:** 337-339.
11. Kuhns MC, McNamara AL, Perrillo RP, Cabal CM, Campbel CR. Quantitation of hepatitis B viral DNA by solution hybridization: comparison with DNA polymerase and hepatitis B e antigen during antiviral therapy. *J Med Virol* 1989; **27:** 274-281.
12. Korenman J, Baker B, Waggoner J, Everhart JE, Di Bisceglie AM, Hoofnagle HJ. Long-term remission of chronic hepatitis B after alpha-interferon therapy. *Ann Intern Med* 1991; **114:** 629-634.
13. Brook MG, Karayiannis P, Thomas HC. Which patients with chronic hepatitis B virus infection will respond to alpha-interferon therapy? A statistical analysis of predictive factors. *Hepatology* 1989; **10:** 761-763.
14. Quiroga JA, Mora I, Porres JC, Carreno V. Elevation of 2',5' -oligoadenylate synthetase activity and HLA-I associated beta 2-microglobulin in response to recombinant interferon-gamma administration in chronic HBeAg-positive hepatitis. *J Interferon Res* 1988; **8:** 755-763.
15. Bodsworth N, Donovan B, Nightingale BN. The effect of concurrent human immunodeficiency virus infection on chronic hepatitis B: a study of 150 homosexual men. *J Infect Dis* 1989; **160:** 577-582.
16. Lok AS-F, Lai C-L, Leung EK-Y. Interferon antibodies may negate the antiviral effects of recombinant α-interferon treatment in patients with chronic hepatitis B virus infection. *Hepatology* 1990; **12:** 1266-1270.
17. Mayet WJ, Hess G, Gerken G, *et al.* Treatment of chronic type B hepatitis with recombinant alpha-interferon induces autoantibodies not specific for autoimmune chronic hepatitis. *Hepatology* 1989; **10:** 24-28.
18. Renault PF, Hoofnagle JH. Side-effects of alpha interferon. *Semin Liver Dis* 1989; **9:** 273-277.
19. Porres JC, Carreno V, Ruiz M, Marron JA, Bartolome J. Interferon antibodies in patients with chronic HBV infection treated with recombinant interferon. *J Hepatol* 1989; **8:** 351-357.
20. Antonelli G, Currenti M, Turriziani O, Riva E, Dianzani F. Relative frequency of nonneutralizing antibodies to interferon (IFN) in hepatitis patients treated with different IFN-α preparations. *J Infect Dis* 1991; **163:** 882-885.
21. Dianzani F. Biological basis for the clinical use of interferon. *Gut* 1993; **34:** suppl, S74-S76.
22. Garcia de Ancos JL, Roberts DA, Dusheiko GM. An economic evaluation of the costs of α interferon treatement of chronic active hepatitis due to hepatitis B or C virus. *J Hepatol* 1990; **11:** S11-S18.
23. Brunetto MR, Oliveri F, Rocca G, *et al.* Natural course and response to interferon of chronic hepatitis B accompanied by antibody to hepatitis B e antigen. *Hepatology* 1989; **10:** 198-202.

24. Fattovich G, Farci P, Rugge M, *et al.* A randomized controlled trial of lymphoblastoid interferon-α in patients with chronic hepatitis B lacking HBeAg. *Hepatology* 1992; **15:** 584-589.
25. Lok AS, Lai CL, Wu PC, Lau JY, Leung EK, Wong LS. Treatment of chronic hepatitis B with interferon: experience in Asian patients. *Semin Liver Dis* 1989; **9:** 249-253.
26. Milich DR, Jones JE, Hughes JL, Price J, Raney AK, McLachlan A. Is a function of the secreted hepatitis B e antigen to induce immunologic tolerance *in utero*. *Proc Natl Acad Sci USA* 1990; **87:** 6599-6603.
27. Ruiz-Moreno M, Jimenez J, Porres JC, Bartolome, J, Moreno A, Carreno V.A controlled trial of recombinant interferon-alpha in Caucasian children with chronic hepatitis B. *Digestion* 1990; **45:** 26-33.
28. Kato N, Hijikata M, Nakagawa M, *et al.* Molecular structure of the Japanese hepatitis C viral genome. *FEBS Lett* 1991; **280:** 325-328.
29. Davis GL. Interferon treatment of viral hepatitis in immunocompromised patients. *Semin Liver Dis* 1989; **9:** 267-272.
30. Todo S, Demetris AJ, Thiel D, Teperman L, Fung JJ, Starzl TE. Orthotopic liver transplantation for patients with hepatitis B virus-related liver disease. *Hepatology* 1991; **13:** 619-626.
31. Rakela J, Wood JR, Czaja AJ, *et al.* Long-term versus short-term treatment with recombinant interferon alfa-2a in patients with chronic hepatitis B: a prospective, randomized treatment trial. *Mayo Clin Proc* 1990; **65:** 1330-1335.
32. Lisker-Melman M, Webb D, Di Bisceglie AM, *et al.* Glomerulonephritis caused by chronic hepatitis B virus infection: treatment with recombinant human alpha-interferon. *Ann Intern Med* 1989; **111:** 479-483.
33. Aoki-Sei S, O'Brien MC, Ford H, *et al. In vitro* inhibition of hepatitis B virus replication by 2',3' -dideoxyguanosine, 2',3' -dideoxyinosine, and 3' -azido-2',3' -dideoxythymidine in 2.2.15 (PR) cells. *J Infect Dis* 1991; **164:** 843-851.
34. Marcellin P, Ouzan D, Degos F, *et al.* Randomized controlled trial of adenine arabinoside 5'-monophosphate in chronic active hepatitis B: comparison of the efficacy in heterosexual and homosexual patients. *Hepatology* 1989; **10:** 328-331.
35. Ponzetto A, Fiume L, Forzani B, *et al.* Adenine arabinoside monophosphate and acyclovir monophosphate coupled to lactosaminated albumin reduce woodchuck hepatitis virus viremia at doses lower than do the unconjugated drugs. *Hepatology* 1991; **14:** 16-24.
36. Fiume L, Betts CM, Busi C, *et al.* The pathogenesis of vacuoles produced in rat and mouse liver cells by a conjugate of adenine arabinoside monophosphate with lactosaminated albumin. *J Hepatol* 1992; **15:** 314-322.
37. Fattovich G, Brollo L, Pontisso P, *et al.* Levamisole therapy in chronic type B hepatitis. Results of a double randomized trial. *Gastroenterology* 1986; **91:** 692-696.
38. Fattovich G, Giustina G, Brollo L, *et al.* Therapy for chronic hepatitis B with lymphoblastoid interferon-α and levamisole. *Hepatology* 1992; **16:** 1115-1119.
39. Mutchnick MG, Appelman HD, Chung HT, *et al.* Thymosin treatment of chronic hepatitis B: a placebo-controlled pilot trial. *Hepatology* 1991; **14:** 409-415.
40. Perrillo RP, Schiff ER, Davis GL, *et al.* A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. The Hepatitis Interventional Therapy Group. *N Engl J Med* 1990; **323:** 295-301. (Comment in: *N Engl J Med* 1990; **323:** 337-339.)
41. Dept of Health and Human Services. Transcript of FDA antiviral advisory committee meeting on FIAU toxicity (abstract). Services, 1993.
42. Chang C-N, Skalski V, Hua Zhou J, Cheng YC. Biochemical pharmacology of (+)- and (-)-2',3' -dideoxy-3' -thiacytidine as anti-hepatitis B virus agents. *J Biol Chem* 1992; **267:** 22414-22420.
43. Chan S-W, Simmonds P, McOmish F, *et al.* Serological responses to infection with three different types of hepatitis C virus. *Lancet* 1991; **338:** 1391.
44. McOmish F, Chan S-W, Dow BC, *et al.* 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* 1993; **33:** 7-13.
45. McFarlane IG, Smith HM, Johnson PJ, Bray GP, Vergani D, Williams R. Hepatitis C virus antibodies in chronic active hepatitis: pathogenetic factor or false-positive result? *Lancet* 1990; **334:** 754-757.
46. Schvarcz R, von-Sydow M, Weiland O. Autoimmune chronic active hepatitis: changing reactivity for antibodies to hepatitis C virus after immunosuppressive treatment. *Scand J Gastroenterol* 1990; **25:** 1175-1180.
47. Lenzi M, Johnson PJ, McFarlane IG, *et al.* Antibodies to hepatitis C virus in autoimmune liver disease: evidence for geographical heterogeneity. *Lancet* 1991; **338:** 277-280.
48. Onji M, Kikuchi T, Michitaka K, Saito I, Miyamura T, Ohta Y. Detection of hepatitis C virus antibody in patients with autoimmune hepatitis and other chronic liver diseases. *Gastroenterol Jpn* 1991; **26:** 182-186.
49. Mishiro S, Hoshi Y, Takeda K, *et al.* Non-A, non-B hepatitis specific antibodies directed at host-derived epitope: implication for an autoimmune process. *Lancet* 1990; **336:** 1400-1403.
50. Davis GL, Balart LA, Schiff ER, *et al.* Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. *N Engl J Med* 1989; **321:** 1501-1506.
51. Di Bisceglie AM, Martin P, Kassianides C, *et al.* Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. *N Engl J Med* 1989; **321:** 1506-1510.
52. Chayama K, Saitoh S, Arase Y, *et al.* Effect of interferon administration on serum hepatitis C virus RNA in patients with chronic hepatitis C. *Hepatology* 1991; **13:** 1040-1043.
53. Varagona G, Brown D, Kibbler H, *et al.* Response, relapse and retreatment rates and viraemia in chronic hepatitis C treated with α-2b interferon. A phase III study. *Eur J Gastroenterol Hepatol* 1992; **4:** 707-712.
54. Kakumu S, Arao M, Yoshioka K, *et al.* Recombinant human alpha-interferon therapy for chronic non-A, non-B hepatitis: second report. *Am J Gastroenterol* 1990; **85:** 655-659.
55. Iino S, Hino K, Kuroki T, Suzuki H, Yamamoto S. Treatment of chronic hepatitis C with high-dose interferon α-2b: a multicenter study. *Dig Dis Sci* 1993; **38:** 612-618.
56. Schvarcz R, Glaumann H, Weiland O, Norkrans G, Wejstal R, Fryden A. Histological outcome in interferon alpha-2b treated patients with chronic posttransfusion non-A, non-B hepatitis. *Liver* 1991; **11:** 30-38.
57. Okamoto H, Sugiyama Y, Okada S, *et al.* Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. *J Gen Virol* 1992; **73:** 673-679.
58. Okamoto H, Kurai K, Okada S-I, *et al.* Full-length sequence of a hepatitis C virus genome having poor homology to reported isolates: comparative study of four distinct genotypes. *Virology* 1992; **188:** 331-341.
59. Yamada G, Takahashi M, Tsuji T, Yoshizawa H, Okamoto H. Quantitative HCV RNA and effect of interferon therapy in chronic hepatitis C. *Dig Dis Sci* 1992; **37:** 1926-1927.
60. Krawczynski K, Beach MJ, Bradley DW, *et al.* Hepatitis C virus antigen in hepatocytes: immunomorphologic detection and identification. *Gastroenterology* 1992; **103:** 622-629.
61. Czaja AJ, Taswell HF, Rakela J, Schimek CM. Frequency and significance of antibody to hepatitis C virus in severe corticosteroid-treated autoimmune chronic active hepatitis. *Mayo Clin Proc* 1991; **66:** 572-582.
62. Fernandez H, Banks G, Smith R. Ribavirin: a clinical overview. *Eur J Epidemiol* 1986; **2:** 1-14.
63. Reichard O, Andersson J, Schvarcz R, Weiland O. Ribavirin treatment for chronic hepatitis C. *Lancet* 1991; **337:** 1058-1061.
64. Di Bisceglie AM, Shindo M, Fong T-L, *et al.* A pilot study of ribavirin therapy for chronic hepatitis C. *Hepatology* 1992; **16:** 649-654.
65. Rassam S, Dusheiko G. Ribavirin treatment of chronic hepatitis C: a phase I study. Submitted 1992.

Blood transfusion and hepatitis viruses

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Transmission of hepatitis viruses has been recognised as an undesirable effect of blood transfusion since the 1940s, when large outbreaks occurred following inoculation with a yellow fever vaccine which contained pooled human plasma. Further reports followed of jaundice occurring several months after transfusions with blood or plasma.[1] It was also noted in studies in the UK that the incidence of icteric hepatitis increased relative to the number of units transfused.[2]

After the discovery of the Australia antigen in 1965, its recognition as a marker of hepatitis B virus (HBV) infection and its association with post-transfusion hepatitis (PTH), the subsequent introduction of screening tests for this antigen in the early 1970s led to a marked decrease in the incidence of PTH. However, despite increasingly sensitive testing methods for hepatitis B surface antigen (HBsAg), as it subsequently became designated, viral hepatitis was still considered the commonest lethal complication of blood transfusion.[2] It was clear that there were still a number of cases of PTH that were due neither to hepatitis A virus nor to HBV, and the term 'non-A, non-B hepatitis' (NANBH) was coined.

The introduction of molecular techniques enabled clones to be derived from the genome of an agent associated with transfusion-transmitted NANBH,[3] and the proteins derived from these clones were then used to develop an enzyme-linked immunosorbent assay (ELISA)[4] to detect antibodies to this virus, now termed hepatitis C virus (HCV). This ELISA is now used in most developed countries to screen for HCV antibodies.

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