Reviews in Medicine

Liver disease

Stephen D. Ryder and Roger Williams

Institute of Liver Studies, King's College Hospital and School of Medicine and Dentistry, Bessemer Road, London SE5 9PJ, UK

Introduction

In the last year there have been many significant developments in clinical hepatology covering the fields of viral hepatitis, portal hypertension, hepatic cancer and liver transplantation. This review cannot be comprehensive and therefore areas have been highlighted where there have been recent important clinical changes or major developments in basic science where our understanding of disease processes has advanced.

Viral hepatitis

There have been many changes in our understanding of the clinical patterns and science of viral hepatitis. There are at least five major types of primary hepatotropic hepatitis viruses which cause significant liver disease in humans. There are many other viruses such as cytomegalovirus, Epstein–Barr virus and herpes simplex which can cause a hepatitis, but this feature is usually markedly less prominent than other system involvement with the infection. We have therefore confined our review to primary hepatotropic viruses.

Hepatitis A virus (HAV)

HAV as an epidemic-causing, faeco-orally transmitted agent was known to exist for many years prior to its final isolation in 1973.¹ HAV is an RNA virus and is classified as a picornavirus, although it has differences compared to the well-studied four genera of this virus family.² HAV still represents a major health problem, particularly in the developing world where sanitation is poor and mass epidemics still occur.³ In Western populations mass outbreaks are rare but HAV infection is increas-

ingly prevalent among high-risk groups, such as homosexual men.⁴ While HAV usually causes a minor or unnoticed illness in children and young adults, it can cause serious illness, including fulminant hepatic failure, in adults and particularly in patients over the age of 60.⁵ In this older age group a significant mortality and morbidity is seen with acute HAV infection and almost all complications are more frequent. Worldwide, less than 5% of the cases of HAV are recognized clinically.^{6,7} In the recent major outbreak in Shanghai, China, approximately one third of those shown to be serologically positive for acute HAV infection were asymptomatic, and only 20% had overt clinical hepatitis.⁸

Hepatitis A is a self-limiting illness which never causes chronic liver disease. Atypical systemic manifestations such as sinus bradycardia and transient T-wave changes on electrocardiography, arthralgia and palpable splenomegaly do occur but in less than 15% of cases.⁵ Prolonged cholestasis and the occurrence of a relapsing course are well described but rare in HAV infection.^{9,10} minor coagulation defects and depressed fibrinogen levels are, in contrast, relatively frequent but have little clinical significance.^{5,11}

Prevention of infection

There is no effective form of therapy for acute HAV infection, partly because at the time of presentation viral replication usually has already ceased and efforts have therefore been directed at providing immune-mediated protection from infection. Until recently this was only achieved by the use of passive immunization with serum immunoglobulin preparations. These are well known to be effective in both pre-exposure and post-exposure prophylaxis against HAV infection¹² but there are clear drawbacks to the use of this preparation. The protection offered by a single dose of immunoglobulin is only 3–6 months and the product itself is a limited resource derived from human plasma. The development of killed hepatitis A vaccine therefore represents a major improvement in pro-

Correspondence: Roger Williams, C.B.E., Institute of Liver Studies, King's College Hospital, Denmark Hill, London SE5 9RS, UK.

phylactic therapy for high-risk groups.¹³ An inactivated hepatitis A vaccine now licensed for use in Europe has shown seroconversion rates of 95.7% 4 weeks after a single dose of vaccine and 99.8% after a second dose given one month later.¹⁴ The current recommendation is that three doses are given over 6 months and following this high levels of antibody can be demonstrated.¹⁵ The levels of antibody found in this study were up to 25 times higher than those found in passively immunized subjects (who are known to have protective levels of antibody). This suggests that immunity to HAV will persist for many years following immunization with killed vaccine. It has also been shown that killed vaccine given as a single dose, is protective against hepatitis A infection in humans.¹⁶

Major advances have also been made in detection of patients without immunity to HAV. Salivary antibody tests are now available and have been shown to be both accurate and acceptable to patients.^{17,18} The development of these tests suggests that not all travellers to areas with endemic HAV should be immunized, it is cost effective to screen for antibodies and to immunize only those at risk¹⁹ (Table I). It would seem sensible to offer killed active vaccination to at-risk groups wherever time permits and to reserve passive immunization for patients who require immediate protection. Attenuated HAV vaccine and recombinant vaccines are being investigated.^{20–22}

Hepatitis B virus (HBV)

HBV infection remains one of the major health problems in the world today. It is thought that there are approximately 300 million chronic HBsAg (hepatitis B surface antigen) carriers worldwide²³ and The World Health Organization estimates that 40% of them will eventually die of chronic liver disease and/or hepatocellular carcinoma (HCC).²⁴

HBV is a DNA virus and its genome has now been well characterized. It consists of four overlapping open reading frames, S/pre-S, C, P, and X (Figure 1). The S/pre-S region of the genome codes for the proteins of the viral envelope.²⁵ Cytokines or their receptors appear to be important in the replication of HBV,²⁶ pre-S sequences and the IL6 receptor are involved in the binding of virions to a hepatocyte cell surface which may be the first step in infection of the cell.^{26,27} Both HBcAg (hepatitis B core antigen) and HBeAg (hepatitis B e antigen) are encoded by the C gene, which includes a pre-C region.^{27,28} Existing evidence suggests that HBcAg is the major determinant responsible for immunogenicity.^{29–34} When pre-C is transcribed, the core protein can also be targeted to the endoplasmic reticulum, where it is subsequently cleaved and HBeAg is secreted from the cell into the blood.³⁵ The presence of HBeAg in the blood is therefore an indicator of ongoing viral replication. The X protein may also be related to HBV replication.^{36–38}

There are an increasing number of different mutant HBVs recognized,^{39,40} usually resulting from single point mutations, which give rise to different clinical patterns of serological response and disease. The pre-core mutant, initially identified in Mediterranean populations, produces

Table 1 Groups recommended to have antibody screening for HAV infection and immunization (US guidelines)

Child care centre workers
Institutionalized patients
Medical personnel
Travellers to endemic areas
Contacts of cases
Food handlers
Prison inmates
Military personnel
Homosexual men

Figure 1 Structure of the HBV genome. The positions of the four open reading frames (ORFs) known to produce HBV proteins are shown in open boxes. The relative positions of the enhancer element (ENH), glucocorticoid responsive element (GRE), and the direct repeats (DR1 and DR2) are also shown (+) and (–) denotes the positive and negative strands. Small, solid arrows show the 5' ends of the three major virus RNA transcripts of 2.1, 2.4, 3.5 kb.

infected patients who are serum HBeAg negative but serum HBV-DNA positive. They often have severe progressive chronic hepatitis. This HBV variant has a single point mutation in the pre-C sequence which generates a translational stop codon.^{41,42} The absence of pre-C protein excludes the production of HBeAg and this explains the persistence of HBV replication in some anti-HBe-positive patients.⁴³ Use of the polymerase chain reaction (PCR) allows the detection of very low levels of DNA in serum and this has become an increasingly important clinical tool in the face of these mutant viruses which do not produce the usual serum markers. With this technique, HBV sequences have been detected in a proportion of HBsAg seronegative patients with chronic liver disease, including primary hepatocellular carcinoma.⁴⁴ The pre-core mutants have been associated with fulminant hepatitis in Japan and Israel⁴³ which raises the possibility that mutant HBV may alter the host immune response and therefore the clinical course of the disease. Surface gene mutations have been described⁴⁵ but appear relatively rare and may be related to the increased use of hepatitis B vaccination as these variants appear to be able to replicate despite vaccine-induced immunity.⁴⁵

Pathogenesis of liver damage

Infection with HBV results in destruction of liver cells although the existence of an HBV carrier state without evidence of liver damage makes it unlikely that HBV is directly cytopathic in man.⁴⁶ It appears that nucleocapsid antigen containing hepatocytes form a major T-cell target and that their removal may cause or contribute to permanent liver damage.⁴⁷ Other immune mechanisms clearly play a part but considerably less is known about these, although liver-specific membrane lipoprotein (LSP) may be a non-T-cell mediated immune target.⁴⁸

The major determinant of the clinical course of the disease is the method of transmission. Parenteral and sexual transmission is the major route in the Western world and this mode of infection results in chronic HBV infection in only approximately 5% of cases. In the developing world the predominant modes of transmission are perinatal and horizontal, thought to be via inoculation of skin or mucus membranes, possibly increased by traditional healing practices.⁴⁹ The majority of infections acquired perinatally result in chronic HBsAg positivity and ongoing viral replication with a very low rate of spontaneous clearance of HBsAg.⁵⁰

Inborn or acquired immune defects, such as other chronic infections⁵¹ have been shown to predispose to chronicity of HBV infection. The

mechanisms of failure of the normal response to HBV infection have been the subject of intensive study. Interferon-alpha production is usually very high in acute infection but greatly suppressed in chronic infection.^{52,53} This raises the possibility that interferon deficiency may predispose to the development of chronic HBV infection. HBV itself can inhibit interferon production *in vitro* which may explain the decreased interferon production observed in this group of patients.^{54,55} Many other immune defects including abnormal natural killer cell function, decreased *in vitro* anti-HBs production^{56,57} and increased production of interleukin 1 and tumour necrosis factor-alpha have been demonstrated in chronic HBV infection^{58,59} but the exact role of these changes in the suppression of the normal response to the virus remains to be elucidated.

Anti-viral therapy

A large array of therapies have been utilized in chronic HBV infection. The only successful therapy to date has been interferon alpha.⁶⁰⁻⁶⁴ However, the success rate of lymphoblastoid and recombinant interferon-alpha in these patients varies from only 20 to 40%. This variable response rate is not due to variable interferon-alpha receptor expression^{65,66} and many host factors have now been identified which predict poor or good responses in an individual patient (Table II).

There are other limitations to the use of interferon alpha; thrombocytopenia, influenza-like syndrome, psychiatric disturbances, thyroid dysfunction⁶⁷ and, recently, haemolysis⁶⁸ have all been described in addition to decompensation of liver disease in cirrhotic patients. Many centres would not treat patients with Child's class B or C cirrhosis as the risk of inducing encephalopathy with therapy appear high.⁶⁹ The treatment of patients with cirrhosis is, however, often the only chance of stopping viral replication as the spontaneous remission rate is low. In this group of patients further liver damage may well be critical and there is little doubt that some patients improve clinically if the HBV infection is rendered non-replicative.⁷⁰ Many centres would therefore treat cirrhotic patients, starting at a low dose of interferon (3 millions units, three times per week) gradually increasing to the maximal tolerated dose. There is no evidence that cirrhotic patients have a lower response rate if they can tolerate the therapy.⁷¹ Interferon alpha therapy may induce new mutations in the virus, one HBsAg positive, anti-e positive patient with replicating HBV showed a change in the viral genome on treatment, with a new mutant virus appearing with two pre-core genome changes.⁷² Many combinations of agents and new agents are currently under trial for

Table II Patient-related determinants that are thought to influence the response to interferon alpha therapy

<i>Likely to respond</i>	<i>Unlikely to respond</i>
Females	Males
Infection acquired in adulthood	Infection acquired in childhood
Low pretreatment HBV-DNA	High pretreatment HBV-DNA
High pretreatment transaminases	Low pretreatment transaminases
Chronic active hepatitis on biopsy	Minimal inflammation on biopsy
Heterosexual	Homosexual
Delta virus negative	Delta virus positive
HIV negative	HIV positive

patients who do not respond to interferon alpha therapy, the deoxynucleoside agents 5ddC and 5-FSddc have been found to be highly active against the virus *in vitro*⁷³ and this type of compound may have promise as a clinical agent.

Hepatitis B immunization

Plasma-derived HBV vaccines were first available in 1981 but their use was limited by cost and the fear of blood-borne disease transmission. Recombinant HBV vaccines became available in 1986 allowing major vaccination programmes to be undertaken for the first time. As experience with these vaccines has grown a number of factors determining response have been established (Table III).

Response to the hepatitis B vaccine may be linked to the major histocompatibility complex and inherited in a dominant fashion.⁷⁴ Vaccine responsiveness is also impaired in immunocompromised individuals, HIV seropositive patients had a seroconversion rate of less than 25% overall⁷⁵ with no subject with a T4 lymphocyte count below 700 cells per microlitre seroconverting after immunization.

Table III Response to HBV vaccination: host factors

Factors predictive of poor HBV vaccine response

Age
Obesity
HIV infection
Immunosuppressive therapy/diseases
Alcohol excess
Chronic disease

Major immunization programmes have been established throughout the world, usually targeting medical personnel and babies born to HBsAg seropositive mothers in most developed countries.^{76,77} In some endemic areas, like Hong Kong and Taiwan, all newborn babies are routinely vaccinated. The World Health Organization has recommended routine HBV vaccination for countries that possess the economic capacity to purchase the vaccine and where the HBV carrier rate exceeds 2.5% of the population. Mass immunization is also being adopted in some developed countries such as the USA because of the failure to reduce the prevalence of HBV infection by targeting only high-risk groups.⁷⁸ The seroconversion rates of all available vaccines is between 80 and 95%. Around 35% of healthy HBV vaccine responders will show a steady decline in anti-HBs titre and this frequently drops below 10 mIU/ml at 5 years. The Immunization Practices Advisory Committee at present does not recommend booster doses for these subjects. However, studies in homosexual men demonstrated that the life-table attack rate of new HBV infections in vaccinated responders was 1.8% annually.^{78,79} Although most of these new infections were subclinical seroconversions, one patient developed clinical hepatitis.⁷⁹ HBV mutants may be responsible for some of the patients who developed HBV infection after successful vaccination⁸⁰ and new HBV vaccines, which include additional antigens such as pre-S1, pre-S2 and possibly HBc, are being developed to attempt to overcome this problem.⁸¹

Hepatitis C virus (HCV)

There has been a recent explosion of interest in, and publications relating to, hepatitis C virus infection. The hepatitis C virus is the predominant cause of

transfusion-associated hepatitis in the UK. It was initially identified independently by two groups^{82,83} and found to be a single-stranded RNA virus of approximately 10,000 nucleotides. The genome of HCV has been sequenced and found to consist of a single open reading frame which is presumably cleaved post-translationally.⁸⁴ Following infection antibodies to HCV take at least 1 to 3 months to appear in the serum.⁸⁵ Antigenaemia is so limited that circulating viral antigen is beyond the limit of detection of most conventional assay techniques.⁸⁴

Currently available serological assays

The first generation diagnostic tests for antibodies to HCV were based on detection of antibodies to the product of the 5-1-1 clone which was combined with overlapping clones to produce a larger viral antigen C100. The major problem with this assay was that this antigen could be absent from the serum of infected individuals for up to 1 year and therefore other determinants were examined. Second generation tests, recombinant immunoblot assay II (RIBA II) detect a panel of antigens, anti-C100-3, anti-5-1-1, and anti-C33 and anti-C22⁸⁶ (Figure 2). Anti-C100-3 and anti-C33 often appear earlier than C100 in acute infection. Antibody to nucleocapsid protein (P22) may be present when anti-C100 is negative.⁸⁷ Using the serological assay for the antibody to non-structural protein of the virus, it has been shown that HCV accounted for 80% of the post-transfusion hepatitis. Screening of blood donors has dramatically reduced the incidence of post-transfusion hepatitis to 3 per 10,000 units in the USA.⁸⁸

There is no doubt that while serological tests have a role in screening of patients at risk they are far from perfect. The polymerase chain reaction provides a highly sensitive means of detecting HCV RNA in the serum and tissue of patients. Primer selection is important and the 5' non-coding region (the least genetically variable) is usually used.⁸⁹ PCR is a better predictor of infectivity than antibody detection⁹⁰ and is positive within a very short period following infection.⁹¹ PCR positivity is very closely correlated with histological evidence of

chronic hepatitis⁹² and positive PCR is highly likely to indicate chronic hepatitis on liver biopsy even in the presence of normal transaminases.

The genome of HCV shows considerable variation, with four major subtypes from Japan, USA and Europe.⁹³ This may have considerable importance in the determination of chronicity and possibly severity of infection, and will have implications for vaccine development.

On the basis of HCV antibody testing of blood donors, approximately 500 million people worldwide are believed to have been infected. The mode of infection is post-transfusion in 25% and about half of all patients have no identified risk factors. The risk to health care workers is substantial with about 10% of needle-stick injuries from HCV-RNA-positive patients resulting in seroconversion.⁹⁴ There is no risk if patients are RNA negative. Body fluids other than blood do not appear to contain the virus,^{95,96} although there are reports of its detection in saliva; dentists and oral surgeons are at increased risk.⁹⁷ The large group of patients with no identified risk factors raise the possible role of sexual transmission but a study of patients with haemophilia suggests that if this happens at all it appears to be a very rare event.⁹⁸ Community acquired HCV is very common in many parts of the world, including Southern Europe, but the exact mode of transmission remains uncertain. It has been suggested that some of these cases may result from the use of dirty needles in immunization procedures in infancy but the degree of potential spread from this source is uncertain.⁹⁹ Vertical transmission also appears a rare event unless the mother is human immunodeficiency virus (HIV) positive as well.

Spectrum of liver disease

The clinical spectrum of liver disease is of chronic persistent hepatitis through chronic active hepatitis to cirrhosis and hepatocellular carcinoma. The period of time from infection to cirrhosis has been estimated from follow-up of patients with defined post-transfusion liver disease and is probably at least 20 years,¹⁰⁰ although some patients may clear the virus.¹⁰¹ The histological picture of HCV infection is relatively characteristic, showing lymphoid aggregates in the portal tracts with usually mild lobular activity and bile duct damage.¹⁰² In the majority of asymptomatic blood donors or patients detected with mildly elevated transaminases, the histological appearance is usually mild chronic active hepatitis, often with some fatty change.

Treatment

Therapy for hepatitis C is at present restricted to alpha-interferon. Treatment with 3 million units

Figure 2 Schematic diagram of HCV proteins indicating the locations of viral proteins which form target antigens for current serological antibody testing.

three times per week reduces transaminases in about 50% of patients but only about half of these have a sustained response at the end of treatment.^{103,104} Longer courses of treatment do not appear to increase the proportion of patients with PCR and histological remission at the end of therapy. Recent evidence shows that patients with cirrhosis rarely have a sustained response to interferon (Tibbs, personal communication) and the risks of treatment in the setting of cirrhosis are greater. Treatment of this group of patients with interferon is therefore not recommended. The only other agent in common use for hepatitis C is ribavirin which has good activity against RNA viruses *in vitro*. In clinical trials it has proved disappointing with a reduction in transaminases during therapy but a prompt return of activity at the end of treatment,¹⁰⁵ its use is also limited by haemolysis at therapeutic dosage. This agent may be more useful in the post-transplant setting.

Autoimmunity and HCV

Hepatitis C infection has been associated with a number of auto-immune phenomena and there has been considerable debate as to the role of HCV infection in autoimmune chronic active hepatitis (CAH). There appears to be a subgroup of patients, usually older males, with autoimmune markers, predominantly anti-liver-kidney-microsomal (LKM1), who test positive for HCV antibodies and have HCV-RNA in the serum.¹⁰⁶ These patients are a subgroup of classical type 2 autoimmune CAH and the overlap may be due to cross-reactivity between the cytochrome p450 antigen recognized by LKM1 and an amino-acid sequence within the hepatitis C genome. Patients with HCV infection tend to have lower titres of anti-LKM1 and anti-GOR antibodies may be more specific for this subgroup.¹⁰⁷ It is important to make the diagnosis by PCR for HCV-RNA as treatment of patients with true autoimmune CAH with interferon results in worsening of the condition.¹⁰⁸ There is no evidence that patients with type 1 (anti-smooth muscle antibody positive) autoimmune CAH in the UK population have co-infection with hepatitis C virus, initial screening with the first generation antibody tests produced many positive results in this group but this may well be an artifact due to the high level of immunoglobulins.¹⁰⁹ It has proved impossible to detect HCV-RNA in patients with type 1 CAH who have positive first or second generation antibody tests.¹¹⁰

It is increasingly recognized that HCV infection causes more than liver disease. Mixed essential cryoglobulinaemia has been shown to be due to HCV infection in a high proportion of cases¹¹¹ and the cryoprecipitates in this condition are composed of HCV virions and antigen-antibody com-

plexes.¹¹² Patients with Sjogren's syndrome have a high incidence of HCV infection¹¹³ and renal involvement in HCV infection can occur either as a result of cryoglobulin deposition or IgA deposition.

Hepatitis D (HDV) and E virus (HEV)

HDV infection in the non-transplant setting only occurs in patients with prior or simultaneous HBV infection as HDV requires HBsAg for infection and propagation.¹¹⁴ HDV is able to replicate independently of HBV.^{115,116} HDV probably causes liver damage by a direct cytopathic action¹¹⁷ but immune-mediated mechanisms are also likely to be important in progression of the liver damage. A form of liver-kidney microsomal autoantibody similar to that found in a subset of autoimmune chronic active hepatitis has been described in patients with chronic HDV infection, but its significance is unclear.¹¹⁸ Recent studies in transplanted patients have demonstrated subclinical infection with HDV in the absence of replicating HBV.¹¹⁹

Hepatitis E virus is a faeco-orally transmitted agent which has been associated with epidemics in Asia and Africa. There is a particularly high mortality rate among pregnant women.¹²⁰ Enzyme-linked immunosorbent assay testing is now available for both IgG and IgM anti-HEV antibodies and is a useful diagnostic test for acute (IgM) and past infection.¹²¹ At present, there is no vaccine available and commercially available immunoglobulins do not provide immunity¹²² presumably because the populations these products are derived from have a very low incidence of HEV infection.

Metabolic liver disease

Haemochromatosis

Genetic haemochromatosis (GH) is a common genetic disease with an estimated frequency of the gene in the UK population of 10%, with 3% of the population affected. The basic defect in haemochromatosis is unknown, but the condition is characterized by increased iron absorption and deposition in various body tissues. In the liver, GH causes inflammation, fibrosis, cirrhosis and hepatocellular carcinoma.

In 1976 the strong linkage which exists between HLA-A and the disease was first described¹²³ and subsequently it has been confirmed that the gene for GH lies on the short arm of chromosome 6 (6p) in close proximity to and in linkage disequilibrium with HLA-A. This linkage is sufficiently strong to

allow HLA haplotypes to be used to determine with a high degree of accuracy, family members likely to be affected (Figure 3). Siblings of a proband who share both HLA-A and B haplotypes are putative homozygotes and therefore at high risk.¹²⁴ A large array of microsatellite markers have now been described¹²⁵ which allow intensive study of a 2,000 kilobase region around HLA-A and should allow final location of the gene to be established.

Alpha-1-antitrypsin deficiency

The genetics and cell biology of alpha-1-antitrypsin (α -1-AT) have answered many important questions about the mechanism of disease caused by this genetic syndrome. α -1-AT is an acute phase protein whose biological function is that of a protease inhibitor which protects elastin fibres in the lung from degradation by elastase. There is a single locus for the α -1-AT gene on chromosome 14 but the molecule itself exhibits extensive heterogeneity with more than 60 phenotypes identified. The inheritance is autosomal co-dominant. The pro-

tease inhibitor (Pi) system is used to classify variants on the basis of electrophoretic mobility on isoelectrical focusing and the usual α -1-AT phenotype, PiZ, is characterized by circulating α -1-AT levels of about 10% of normal. An amino-acid substitution in the molecule is responsible for a secretory defect with accumulation of protein within the endoplasmic reticulum in the liver.¹²⁶ Lung disease is strongly related to the level of α -1-AT in the plasma but liver disease correlates with intracellular accumulation of α -1-AT. Liver injury occurs in α -1-AT phenotypes associated with the intracellular accumulation of PAS-positive material but not in those phenotypes where no accumulation is seen.^{127,128} The severity of hepatocellular involvement is variable and is worsened by the formation of liver aggregates at times of pyrexia and stress, and avoidance of these factors forms a major initial step in therapy.¹²⁹ The biological basis of the relationship of temperature and liver damage was explained fully in a recent study¹³⁰ which demonstrated the molecular pathway underlying α -1-AT accumulation. A point

Figure 3 HLA-A and B haplotypes of a family with genetic haemochromatosis. The index case presented with arthropathy, hepatomegaly and glycosuria. Case 2 was asymptomatic but had identical HLA markers and grade 4 siderosis on liver biopsy. The two other family members with some degree of iron loading are heterozygotes and have increased hepatic iron but have no significant disease and remain well. HLA typing is of considerable use in determining individual risk and need for liver biopsy. (Adapted from Bomford, A.B., *Lancet* 1977, by kind permission of the Editor).

mutation results in an amino-acid substitution at a crucial site in the α -1-AT molecule. The Z insertion in α -1-AT results in a unique molecular interaction between the reactive centre loop of one molecule and the gap between the A sheet of another, this reaction occurs rapidly at higher temperatures than normal, resulting in polymerization of α -1-AT molecules and thus explaining the relationship between fever and worsening liver disease (Figure 4).

Other than simple measures to avoid fever there is no other therapy which has been shown to be effective in liver α -1-AT, although replacement therapy may help lung disease. Drugs which increase plasma levels of α -1-AT such as tamoxifen and danazol work by stimulation of synthesis and can worsen liver disease by increasing accumula-

tion. Liver transplantation remains the solitary therapy for advanced liver disease, this procedure may not totally correct the genetic defect as the gene is expressed in several extrahepatic tissues (macrophages and monocytes), although chimerism and the experience with other metabolic diseases (reviewed later) give hope in this respect. It is anticipated that gene therapy may offer future correction of the underlying defect.

Portal hypertension

Portal hypertension appears to be the result of systemic arterial vasodilatation¹³¹ with the primary abnormality occurring in the non-splanchnic circulatory bed which leads to plasma volume expansion and an increase in cardiac index.¹³² This has stimulated the study of a large number of endogenous vasodilator substances but recent interest has centred around the endothelium-derived relaxing factor nitric oxide.¹³³ It has been postulated that high levels of circulating endotoxin found in cirrhotic patients may induce nitric acid synthetase which increases plasma levels of nitric oxide and produces arterial vasodilatation.¹³⁴ It has been demonstrated in animal models that antagonists of nitric acid synthetase can reverse the haemodynamic parameters seen in portal hypertension,¹³⁵ although the underlying pathophysiology and mechanism of this response is disputed.^{135,136} Considerably more evidence needs to be assessed before clinical studies can be undertaken as L-mono methyl arginine (the nitric oxide synthetase inhibitor used in these studies) increased mortality in rats given endotoxin.¹³⁷ The clinical significance of portal hypertension is the mortality and morbidity associated with bleeding oesophageal varices and ascites. Management of oesophageal varices is to attempt to prevent the first bleed (primary prevention) or to prevent re-bleeding (secondary prevention). The ability of clinical and endoscopic criteria to predict patients with cirrhosis who will bleed from varices remains poor, with a substantial proportion of patients without the high-risk criteria of large varices, fundal varices or red spots on varices, suffering an index bleed.¹³⁸⁻¹⁴⁰ Measurement of the hepatic venous pressure gradient has been shown to have a predictive value for survival in patients with oesophageal varices, the higher the pressure the worse the prognosis, with a pressure of 12 mmHg being a reasonable discriminant value.¹⁴¹

Beta-blockers have been shown to reduce the incidence of the first bleed¹⁴² but do not improve outcome in most trials as the dominant influence on survival is liver function.¹⁴³ The lack of any survival advantage in many studies has resulted in the use of sclerotherapy only for bleeding varices and not for

Figure 4 A diagrammatic representation of the A-sheet of α -1-anti-trypsin. The reactive centre loop (shaded) is mobile and can hinge on Glu 342 to fold back into a gap in the A-sheet between strands 3 and 5 to form strand 4 as shown. Disruption of this folding by the Z mutation Glu 342 $\rightarrow$ Lys or opening of the A-sheet by mild denaturation allows instead the insertion of the loop of a second molecule to give loop-sheet polymerization. This polymerization is prevented by an excess of the free BC13 peptide with a sequence homologous to the loop (shaded), which preferentially inserts into the S₄ position. (Reproduced from Lomas *et al.*, *Nature* 1993, 357: 606 with kind permission.)

primary prophylaxis.¹⁴⁴ There is increasing evidence that combining a beta-blocker with a nitrate may be more effective in lowering portal pressure¹⁴⁵ but it is not yet known if this effect results in clinical benefit for the patient.

Endoscopic sclerotherapy has been the mainstay of prevention of rebleeding from oesophageal varices but the recent development of oesophageal variceal ligation appears to be an alternative therapy which may have some advantages.¹⁴⁶ The technique involves placing of small rubber bands onto the variceal column using an endoscopic release technique (Figure 5). It appears that this technique requires fewer endoscopy sessions to produce obliteration of oesophageal varices and that the risk of rebleeding is less.¹⁴⁷ Overall mortality, however, is identical in patients treated by each technique, which reflects the previous experience with all methods of treatment for varices where degree of liver dysfunction is the major prognostic factor.

Non-endoscopic methods of treating oesophageal variceal haemorrhage continue to be developed. Intravenous vasopressin and transdermal nitroglycerin have been used in this situation for many years and are known to be effective.¹⁴⁸ This mode of treatment has major side effects and other agents such as somatostatin have been extensively studied. Somatostatin and octreotide (a long-acting analogue of somatostatin) are effective in the control of acute variceal bleeding; data suggest that somatostatin as sole therapy is somewhat more effective than vasopressin,¹⁴⁹ with 82% versus 72% of patients stopping bleeding and appears to be comparable in effectiveness to injection sclerotherapy¹⁵⁰ with few significant side effects. Even though it seems that somatostatin is probably slightly more effective than vasopressin as medical therapy for varices, the costs of therapy are considerably greater. It is not yet known if the combination of somatostatin and injection sclerotherapy produces further benefit.

Figure 5 Oesophageal variceal band ligation. The varix is sucked into the end of the banding apparatus and the band released. This then strangulates the varix. (Reproduced from Stiegmann & Yamamoto, *Gastro Endoscopy Clinics of North America*, Vol. 2(1), 1992 with kind permission.)

As treatment for oesophageal varices is frequently highly effective, non-oesophageal sites of portal hypertension-related haemorrhage are being seen with greater frequency, with gastric varices accounting for up to 20% of variceal bleeding. High lesser-curve varices may be treated by sclerotherapy and some success has been achieved injecting the tissue adhesive bucrylate, but is usually ineffective if the patient has had prior oesophageal sclerotherapy.¹⁵¹ As a temporary measure balloon tamponade with a Sengstaken–Blakemore tube will usually control haemorrhage but at the expense of a high rate of complications such as aspiration, oesophageal ulceration and perforation.¹⁵² The management of bleeding gastric varices was previously restricted to surgery with shunting or gastric devascularization but the development of transcutaneous intrahepatic portosystemic shunting has provided another therapeutic avenue. This procedure is fully discussed later.

Portal hypertensive gastropathy is a common finding in patients with cirrhosis and is characterized by an increased gastric blood flow.¹⁵³ Portal hypertensive gastropathy has been shown to worsen with injection sclerotherapy and ablation of oesophageal varices.¹⁵⁴ Treatment is generally unsatisfactory and is usually with vasopressin and octreotide. Rectal varices¹⁵⁵ are well recognized and rarely give major clinical problems. Injection therapy can be used to good effect. Portal hypertensive colopathy¹⁵⁶ has also been described. It seems that any part of the gastrointestinal tract can be involved by portal hypertensive vascular changes. The management of these rare problems is usually medical.

Transcutaneous intrahepatic portosystemic shunt

A major development in the management of portal hypertension has occurred with the advent of transcutaneous intrahepatic portosystemic shunts (TIPS). This was a development of prior transjugular techniques developed for embolization of gastric and oesophageal varices.^{157,158} The first description of an intrahepatic shunt using a balloon catheter was in 1982¹⁵⁹ but the initial results were poor as the dilated tract through the liver substance quickly closed resulting in recurrent variceal bleeding.¹⁶⁰ The next major step was the development of expandable vascular stents by Palmaz in 1985 which provided the means to keep the shunt tract patent. The first TIPS procedure was undertaken in 1988.¹⁶¹ Technical development and refinements have continued, and TIPS has now been widely used and some information about its clinical usefulness is available.

The technique of placement^{162–164} involves trans-

jugular cannulation of the right or middle hepatic veins using a 50 cm long sheathed needle. When the cannula is accurately placed, the needle is advanced from the sheath into the liver substance, usually under ultrasound guidance. The needle is then slowly withdrawn while aspirating. When blood is drawn back contrast is injected and when it is confirmed that a portal radicle has been entered a guidewire is passed. The catheter is then advanced into the portal vein and the needle withdrawn. The tract is dilated by balloon and the stent is inserted (Figure 6). Two types of stent are commonly used, the Palmaz stent which is a balloon expandable stent which can be dilated in stepwise fashion from 7 to 14 mm and the Wallstent which is essentially an expandable stent which has a fixed internal diameter when expanded. The advantages of the Palmaz stent are that it is easily visible on radiological imaging and can be dilated further if occlusion or inadequacy of the shunt is established.¹⁶⁵ The Wallstent is easier to place and is potentially removable,¹⁶⁶ although in practice this is rarely attempted. The technical advances and increasing expertise allow placement in approximately 90% of patients¹⁶² and the procedure lasts from 30 minutes to 2 hours in experienced hands.¹⁶⁷ Complications of the procedure are infrequent and consist of bleeding into either biliary tree or abdominal cavity,¹⁶⁴ renal failure due to the high-contrast load,¹⁶⁷ migration of stents¹⁶⁸ into pulmonary or systemic circulation which usually produce few symptoms. The only catastrophic complication is extrahepatic cannulation of the portal vein and stent placement which leads to rapid exsanguination into the abdominal cavity.

The haemodynamic effects of TIPS is related to the size of the shunt established. Using that Palmaz stent with an internal diameter of 7–13 mm, the drop in portal pressure is usually to about 50% of the initial pressure¹⁶⁹ and in the majority of patients the portal to hepatic vein pressure gradient was reduced to below 12 mmHg,¹⁶³ the threshold for variceal bleeding. There is no doubt that TIPS placement stops variceal bleeding in all patients where it can be placed, providing the shunt remains patent. TIPS also improves ascites and pilot studies have shown good results for this indication alone.¹⁷⁰ Variceal rebleeding occurs in about 15% of patients at 1 year, due to stent occlusion by thrombosis or intimal hyperplasia¹⁶⁶ and many of these patients were able to have the stent dilated or replaced. Colour-flow Doppler seems to be the ideal method of monitoring the shunt and detecting early occlusion.¹⁶⁶ Encephalopathy is undoubtedly the major problem with placing TIPS. A total of 25% of patients with TIPS will experience encephalopathy and up to half of these will have persistent cerebral impairment.¹⁷¹ Age and shunt diameter are predictors of post-TIPS encephalo-

Figure 6 Schematic diagram of the transjugular intrahepatic portosystemic stent-shunt procedure. (a) Long-sheathed, curved needle after the right main branch of the portal vein was punctured. A guide wire is introduced through the lumen of the needle. Next, the sheath is moved forward into the portal vein. (b) Implanted metal stent after dilation with a balloon catheter. (Reproduced from Haag & Ochs, *Curr Opin Gastroenterol*, Vol. 9(3) 1993 by kind permission.)

pathy. Child's grade of liver disease is a predictor in young (<60) patients with no Child's A patient developing encephalopathy, but this is not the case in the elderly.¹⁷¹ Long-term survival appears to relate to the severity of underlying liver disease.¹⁶⁷

The place of TIPS in the management of portal hypertension is as yet uncertain and seems sure to be limited by the incidence of encephalopathy and shunt occlusion. Currently, we would recommend its use in patients with severe bleeding problems, especially gastric varices, who are awaiting liver transplantation or those with oesophageal variceal haemorrhage with potential for improvement in synthetic liver function (for example, alcoholic liver disease which may improve with abstinence). Controlled trials against LeVeen shunts for ascites and conventional therapy for bleeding oesophageal varices are required.

Fulminant hepatic failure

Fulminant hepatic failure (FHF) is the term used to describe patients with a rapid clinical progression of their liver disease and is defined as liver failure where the onset of encephalopathy is within 8 weeks of the first symptoms.¹⁷² The most common cause of FHF in the UK is still paracetamol overdose. N-Acetylcysteine acting via increased glutathione, will prevent paracetamol-induced hepatotoxicity if given within 8 hours of the overdose. Its effect is less after this time and it fails to avert the development of severe hepatotoxicity if given more than 15 hours after overdose.¹⁷³ Late treatment of paracetamol-induced FHF with N-acetylcysteine is, however, still of benefit. Patients who develop paracetamol-induced FHF have a better prognosis if they have been given N-acetyl-

cysteine after overdose (even up to 36 hours) in comparison to those who never received it.¹⁷⁴ Treated patients have a lower incidence of renal failure and fewer progress to deep grades of encephalopathy, suggesting that N-acetylcysteine therapy had a later protective effect on other organs. The mechanism of action of N-acetylcysteine at this time is unknown but may be the result of improved tissue oxygen delivery and consumption.¹⁷⁵

Liver transplantation

Orthotopic liver transplantation is now well established as therapy for 'end stage' liver disease where no other therapeutic option exists. The major problem with this mode of therapy is that the supply of donor organs is a limited resource and therefore some degree of selection of candidates will be essential in order to use this resource in order that the maximum benefit possible is derived. Developments in this field include predictive models of underlying liver disease prognosis as well as reports of large series of patients transplanted for a variety of clinical indications allowing some conclusions to be drawn as to patient selection.

One of the most controversial areas in liver transplantation is in patients with alcoholic liver disease. Initial experience in this group of patients was not good,¹⁷⁶ mainly because of the associated cardiac and cerebral disease frequently seen in these patients. With careful pretransplant screening for these complications, the results for transplantation in patients with alcohol related liver disease appear comparable to other patient groups.¹⁷⁷ The major problem in these patients is predicting which patients will return to alcohol abuse. Although intensive psychiatric and psychological assessment may help, there is no good predictor of future abstinence. Investigators from the University of Michigan have developed a prognosis scale¹⁷⁸ to predict further drinking behaviour but this would need extensive investigation before it could ever be used to determine patients suitability for operation. Current policy in the UK remains that patients are required to maintain abstinence from alcohol for 6 months before full consideration for transplantation.

Transplantation for HBV-related liver disease is known to be associated with a high rate of reinfection of the liver graft, frequently with a very poor prognosis. In the King's/Cambridge series of patients transplanted for chronic HBV infection-related liver failure, 84% of the patients became positive for HBsAg at 4–6 weeks post-transplant, probably because of maintained viral replication in extrahepatic sites^{179,180} and the 2 year survival is

only 45%.¹⁸¹ If the patient has replicating HBV, as judged by PCR for HBV-DNA in the serum, the risk of reinfection without any therapy is very high (95%). Even in patients found to be DNA negative, the risk is still substantial and frequently results in fibrosing cholestatic hepatitis, characterized by perisinusoidal bands of fibrosis extending from the portal tracts to surrounding plates of ductal type epithelium, with prominent cholestasis and massive cytoplasmic expression of viral antigens. This syndrome presents as jaundice and a relentless prolongation of prothrombin time, but with only a moderate increase in serum transaminases, leading to loss of the graft within weeks.¹⁸²

Long-term immunoprophylaxis with anti-HBs immunoglobulin combined with active immunization, particularly when the patients are in a non- or low-replicative state at the time of transplantation, has been shown to reduce the rate of graft reinfection and survival figures of 60–70% at 5 years in HBV-DNA-negative patients treated in this way have been reported.^{183,184} Co-infection with the delta virus appears to be associated with less aggressive liver disease after transplantation.¹⁸⁵ Current recommendations are that liver transplantation should be restricted by HBV DNA negative patients who receive prophylactic immunoglobulin postoperatively. This HB-immunoglobulin therapy has not been standardized and the initial perioperative doses used ranged from 500 to 128,000 units. Long-term therapy is adjusted to maintain serum HB-immunoglobulin levels of more than 100 IU/l, and must be carried on indefinitely. It was hoped that the development of monoclonal antibodies to HBV antigens would provide a more effective means of preventing reinfection of the graft but early experience has been that many patients still suffer reinfection but with mutant viruses able to escape the effects of the vaccine.¹⁸⁶ Further developments of vaccines targeting multiple hepatitis B antigens may help.

Chronic liver disease due to hepatitis C virus infection is a major indication for orthotopic liver transplantation in much of the world. As tests for detection of HCV improve the knowledge of the course of HCV infection after transplantation is enhanced. Early studies based on detection of anti-HCV antibodies showed a low rate of HCV positivity (14%) and the overall survival was approximately 88% at 5 years.¹⁸⁷ There is no doubt, however, that many patients with HCV infection do not produce antibodies to the virus post-transplantation.^{188–190} Of patients with pretransplant HCV antibodies, viraemia is present in 95% after transplantation and some patients went on to develop cirrhosis.¹⁹¹ This accords with our own experience, recurrent HCV appears to be common and, although frequently producing only mild liver disease,^{192,193} can run a severe course leading to

cirrhosis and retransplantation.^{191,194} Therapy with interferon has not proved useful in this setting in our patients but a reduction in transaminase levels has been seen in some patients treated with ribavirin (C.J. Tibbs, personal communication), formal studies are awaited.

Transplantation for hepatocellular carcinoma is still a contentious area as in most published series the overall survival rate is very poor. As more data has accumulated it has become apparent that the size of a hepatocellular carcinoma at time of liver grafting appears to be the critical factor; in the King's series, the incidence of tumour recurrence was 0% for tumours up to 4 cm but 70% for large (8 cm or over) and multifocal tumours.¹⁹⁵ Similar figures have been reported from other centres.^{196,197} The excellent results in patients with small tumours make this the treatment of choice in patients with cirrhosis which is not related to hepatitis B virus infection. Some groups have shown an improvement in results of transplantation for large hepatocellular carcinoma using pre- and post-operative chemotherapy¹⁹⁸⁻²⁰⁰ but the King's College series showed no major advantage for neo-adjuvant chemotherapy using doxorubicin²⁰¹ and controlled trials are required.²⁰¹ Patients transplanted for cholangiocarcinoma complicating primary sclerosing cholangitis whether diagnosed pre-operatively or at the time of surgery have a poor prognosis.²⁰² Most transplant units would no longer consider such patients suitable for treatment by liver transplantation and clinical methods of diagnosing cholangiocarcinoma in patients with the multiple biliary strictures characteristic of primary sclerosing cholangitis are needed.

The natural history of patients with primary sclerosing cholangitis has been carefully documented in a multicentre study.²⁰³ Major predictors of survival were serum bilirubin, histological stage of liver disease, age and the presence of splenomegaly. This model is of considerable importance in timing transplantation in this condition.

Biliary complications post-transplantation are a major cause of morbidity and mortality. Evidence is accumulating that two major factors are important in the pathogenesis of strictures, hepatic arterial thrombosis and prolonged cold ischaemia.²⁰⁴ Grafts preserved for 11.5 hours or more in University of Wisconsin solution or in Euro-Collins solution for more than 6.5 hours had a significantly greater incidence of biliary complications. If strictures occur early after transplantation (less than 2 months) this is predictive of a poor outcome.²⁰⁵ Treatment is usually by balloon dilatation or stenting²⁰⁶ but these procedures rarely produce long-term patency, and biliary surgery or retransplantation are often required.²⁰⁵

Immunosuppression for liver transplantation

Cyclosporin A has been a major factor in the improvement of results of liver transplantation during the past decade²⁰⁷ but its nephrotoxicity and other limitations have stimulated a continuing search for alternative agents. FK506 is a macrolide antibiotic which has a totally different structure from cyclosporin A. It is a more potent immunosuppressive agent.²⁰⁸ Patients with early chronic or severe acute rejection could be rescued by changing therapy to FK506²⁰⁹ but patients with well-established chronic rejection did not respond. The side effects of FK506 are generally similar to those of cyclosporin but there appears to be less hypertension and hirsutism.²¹⁰

Tissue typing and liver transplantation

It is well established that matching the graft to the recipients ABO and Rhesus blood groups reduces the risk of hyperacute rejection caused by preformed antibodies. Transplantation across these blood groups is occasionally performed in patients with acute liver failure when an appropriate donor of the same blood group cannot be found. Matching of class I and II major histocompatibility antigens is more controversial, an initial study from Pittsburgh suggested that complex relationship may exist with HLA compatible grafts being protected against graft rejection but having increased susceptibility to recurrence of autoimmune disease.²¹¹ Other studies have shown a trend for a reduction in graft rejection with class I HLA matching.²¹² In a large series of 466 patients receiving first transplants at King's College Hospital and Cambridge, single mismatches of HLA-A or B resulted in a lower incidence of acute and chronic rejection compared to patients with two or three mismatches, and class I matching overall was associated with improved graft survival by reducing both acute and chronic rejection.²¹³

Chimerism

Donor-specific immune tolerance has been and remains the goal in transplantation as the current powerful and effective immunosuppressives used in transplantation work in a non-specific fashion and therefore are associated with major side effects. Transplantation of the liver has important immunological differences from other organs such as kidney or heart: hyperacute rejection by preformed antibodies is considerably less and transplants of skin or other solid organs from the same donor as the liver graft are not rejected, but the same does not apply to subsequent grafts from a different donor. In addition, graft-versus-host disease is less frequent and less severe in liver

transplant recipients than for many other transplanted organs. While many hypotheses have been put forward to explain these observations,^{214–216} migration of host and donor white cells, chimerism, appears to be the major determinant of this immunological tolerance. Following liver transplantation, migration of leucocytes from both donor and graft occur,²¹⁷ a state known as mixed chimerism. In the liver, the epithelial and endothelial components of the liver remain of donor origin but the Kupffer cells, lymphoid cells and dendritic cells in the liver are replaced by cells of host origin.²¹⁸ In the host bone marrow, skin and lymph nodes of women who received grafts from male donors, Y chromosome could be detected.²¹⁹ Metabolic defects such as type IV glycogen storage disease have been shown to be corrected by liver transplantation²²⁰ even though the defect is present in all cells and not just in hepatocytes. The resistance of solid organ allografts to rejection correlates well with the volume of passenger lymphoid cells able to enter the circulation²²¹ and evidence from animal models suggests that only relatively small numbers of donor lymphocytes are required to induce tolerance.²²² Clearly, these findings may have major clinical significance for patients who receive liver grafts as, in theory, if tolerance via chimerism has occurred, then these patients will no longer require such potent immunosuppression and may even be able to have immunosuppression withdrawn altogether. Studies are currently underway to evaluate the degree of chimerism and the specific host–donor immune response.

Hepatocellular carcinoma

The prognosis of HCC is strongly related to the severity of the underlying liver dysfunction, a fact underlined in a recent study of 206 patients who received no therapy.²²³ The overall median survival is only 2.5 months but in patients with good liver function the median survival was over one year. Previous studies have reported similar findings,^{224,225} patients with small tumours can survive considerable periods of time²²⁶ with one, two and three year survival rates of 81, 56 and 21%, respectively. If the severity of underlying liver disease is taken into account, those with good liver function (Child's A) had a 60% survival at 3 years. This means that any potential benefit from therapy has to be carefully assessed as relatively long survival can be seen in selected untreated patients.

It is generally accepted that the only curative options in the treatment of HCC are resection or transplantation. In the western world, few patients are suitable for hepatic resection and results generally have been disappointing. Bismuth *et al.*²²⁷

reported a series of 270 cases where curative resection was possible in only 13% with an operative mortality of 14%. A total of 30% of the survivors developed hepatic parenchymal liver failure and only 40% survived for 16 months. Liver transplantation has been considered previously in this article. Controlled trials of transplantation and resection for small tumours would be useful.

If surgery is not possible, intravenous chemotherapy with doxorubicin has been used and is the most effective single intravenous agent^{228,229} but only 10% of patients respond²³⁰ and median survival in the treated group of the only controlled trials was 3 weeks longer than those given no therapy. Major side effects were seen in 25% of the treated group. Because of this lack of efficacy, methods of targeting chemotherapy have been developed. During its routine use as a radiographic contrast media, it was noted that lipiodol was selectively taken up by hepatocellular carcinomas and was often retained by the tumour for a considerable period – up to 6 weeks (Figure 7).^{231,232} Combining lipiodol with injection of a chemotherapeutic agent directly into the hepatic artery and following this with embolization of the hepatic artery looks a promising mode of therapy^{233,234} with tumour regression demonstrated and comparisons with historical controls showing improved survival. The presence of the lipiodol as a carrier for the chemotherapy appears to improve the effectiveness of the treatment²³⁵ (Table IV).

Our own experience has tended to temper enthusiasm for the procedure. At King's College Hospital only about one third of HCC patients were suitable for chemoembolization (many excluded because of advanced liver disease or portal vein thrombosis). Of the patients treated there was a clear difference in response depending on the initial size of the tumour. Of 12 patients with a tumour of less than 4 cm, seven had a 50% or greater reduction in size of their HCC and there are three survivors at 3 years from diagnosis. Larger tumours did not show such impressive results, 14/55 (25%) showing a significant response but the median survival of 4.7 months is very disappointing considering that the patients with the worst prognosis had already been excluded from treatment.

Percutaneous injection of absolute ethanol into HCC using ultrasound guidance is a recent innovation²³⁶ which has been used in both European and South East Asian centres. The technique is relatively simple to perform with a sedated patient having a 19 or 22G needle introduced directly into the tumour and then a calculated volume of ethanol slowly injected.²³⁷ The treatment is usually undertaken at a single session, with the aim of inducing complete tumour necrosis. There are no controlled trials of this form of therapy against no

Figure 7 (a) CT scan of a patient with HBV-related hepatoma, tumour in R lobe. (b) Hepatic angiogram in the same patient when lipiodol, doxorubicin and gelfoam embolization undertaken. Tumour circulation can be seen. (c) CT scan 3 days after the procedure with retained lipiodol. (d) CT scan 4 months after final embolization, no tumour visible.

Table IV Chemoembolization with and without lipiodol for HCC. From Kasugai *et al.* (1989)²³⁵

Treatment	Number	12 month survival	24 month survival
ADM + TAE	20	41%	6%
ADM + LIP + TAE	25	56%	37%*
CIS + LIP + TAE	52	71%	46%*

ADM = Adriamycin® (doxorubicin hydrochloride); TAE = transcatheter arterial embolization; CIS = cisplatin; LIP = lipiodol; **P*0.05 compared to ADM + TAE.

treatment or chemoembolization. Most published series have been restricted to small (<5 cm) tumours in patients with cirrhosis who are unsuitable for hepatic resection.^{238–240} The largest single series is of 207 Italian patients²⁴¹ with tumours less than 5 cm in diameter and

predominantly with Child's A cirrhosis. This study reports a 3 year survival of 63% which would certainly compare favourably with the expected natural history as described previously where 3 year survival was 25%. The overall survival rates did depend on severity of liver dysfunction but exceptionally good results were reported even for Child's C patients with a 40% 1 year survival. There are considerably less data on the use of ethanol injection for larger HCCs with only one series to date including these patients.²⁴² Larger volumes of ethanol can be injected into big tumours and up to 120 ml have been instilled at a single session in a single tumour with an excellent response as judged by ultrasound (T. Livraghi, personal communication).

The success of alcohol injection for small tumours is similar to that reported from Japan for chemoembolization with lipiodol, with 3 year survival in the range of 40% for patients with small tumours and a mixture of Child's A and B cirrhosis.²⁴² Alcohol injection has a number of poten-

tial advantages, it needs only a single session to produce necrosis whereas chemoembolization may need repeated sessions. This enhances the cost-saving aspect of percutaneous ethanol injection as clearly arteriography and chemotherapeutic agents are expensive. It appears a safe procedure and can be undertaken with an overnight in-patient stay or even as an outpatient procedure. There are, however, potential difficulties, tumour seeding in the needle track has been described²⁴³ as has vascular and bile-duct damage due to the alcohol-mediated tissue necrosis.²⁴⁴ The major disadvantage, however, would seem to be injection in anatomically difficult areas of the liver where obtaining high-quality images may be impossible and technical problems with needle placement may occur. Multicentric disease is also somewhat problematical, although multiple tumour nodules have been successfully treated.²³⁸ Chemoembolization seems theoretically more likely to treat satellite and more distant small secondary lesions not seen on ultrasound effectively but it would always be possible to treat newly visible lesions with percutaneous ethanol injection during follow-up.

Hormonal therapy for HCC is based on the knowledge that the long-term use of oral contraceptives and anabolic steroids are risk factors for HCC^{245,246} and a substantial proportion of HCCs have an increased number of oestrogen and/or androgen receptors.²⁴⁷ The anti-oestrogen drug tamoxifen has been shown to inhibit hepatocyte proliferation *in vitro* and *in vivo*.²⁴⁸ This evidence together with the proven role of tamoxifen in breast and prostate cancers, where high receptor expression is also seen, has led to trials of tamoxifen in HCC. Initial studies showed some potential benefit with a fall in alpha-feto-protein but no obvious tumour shrinkage radiologically^{249,250} and a trial of systemic cytotoxic chemotherapy with and without tamoxifen showed some indication of an increased survival in tamoxifen-treated patients.²⁵¹ The only controlled trial in the literature²⁵² compares two groups of 16 patients receiving tamoxifen and 16 receiving no therapy. Groups were well matched for tumour size and severity of liver disease. Median survival in the tamoxifen-treated group was 5 months versus one month in the control group. Although this trial was small, it does give some encouragement for hormonal manipulation in HCC and it should be remembered that this is at least a very safe form of therapy with no major side effects.

The genetic steps involved in hepatocarcinogenesis have been the subject of intensive study

with a particular focus on mutations within the p53 gene. The p53 tumour suppressor gene plays an essential role in the induction of programmed cell death²⁵³ and mutations in the p53 gene may block this normal cellular function and result in the survival of cells exposed to mutagenic factors. Mutations of the p53 gene are common in many human cancers^{254,255} and are therefore thought to play a major role in the stepwise progression of normal cells toward neoplasia by loss of the 'wild-type' tumour suppressor function.²⁵⁶ Hepatocellular carcinoma is relatively unusual among human cancers in that a number of agents with definite oncogenic potential have been identified, including chronic hepatitis B²⁵⁷ and C infection,^{258,259} cirrhosis²⁶⁰ and aflatoxin B1 exposure.²⁶¹

p53 gene mutations have been found in human HCCs^{262,263} but its relationship to aetiological factors remains uncertain. Bressac *et al.*²⁶² related the high rate of p53 mutations seen in an African population to aflatoxin exposure and suggested that dietary exposure to aflatoxin B1 may induce a particular mutation (codon 249, G-T transversion). The same group expanded their observations by comparing two groups of HCC patients from Southern Africa, from Mozambique and the Transkei who had high and low aflatoxin B1 exposure respectively but a similarly high incidence of hepatitis B virus infection. They found codon 249 mutations to be much more common in the Mozambiquan patients (8/15) than the patients from Transkei (1/12). The situation is, however, far from clear, all the reported G-T transversions at codon 249 were in patients with co-existing hepatitis B virus infection as well as possible aflatoxin exposure. Although point mutations at this codon are not found in many low aflatoxin areas,²⁶⁴ a study of aflatoxin-induced HCC in a primate model²⁶⁵ found a p53 mutation (but not a codon 249 mutation) in 1/4 HCCs in rhesus and cynomolgous monkeys. Other authors have suggested that HBV infection *per se* may produce 249 codon mutations in a South East Asian population with relatively low aflatoxin exposure.^{266,267}

Studies from this unit have shown that p53 protein is detectable in a high proportion of tumour samples from Guangxi, China, where aflatoxin and HBV are common, and also from Brazil, where neither risk factor is thought to be common.²⁶⁸ This interesting observation casts doubt on the potential role of aflatoxin in producing point mutations in the p53 gene, and further studies of p53 gene mutations and aetiological factors are required.

References

Hepatitis A virus

1. Feinslone, S.M., Kapikian, A.Z. & Purcell, R.H. Hepatitis A: detection by immune electron microscopy of a virus like antigen associated with acute illness. *Science* 1973, **182**: 1026–1028.
2. Siegel, G. Virology of hepatitis A. In: Zuckerman, A.J. (ed.) *Viral Hepatitis and Liver Disease*. Alan R. Liss, New York, 1988, pp. 3–7.
3. Halliday, M.L., Kang, L.Y., Zhou, T.K. *et al.* An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. *J Infect Dis* 1991, **164**: 852–859.
4. Centers for Disease Control. Hepatitis A among homosexual men: United States, Canada and Australia. *JAMA* 1992, **267**: 1098–1103.
5. Forbes, A. & Williams, R. Changing epidemiology and clinical aspects of hepatitis A. In: Zuckerman, A.J. (ed.) *British Medical Bulletin: Viral Hepatitis*. Churchill Livingstone, London, 1990, pp. 303–318.
6. Gust, I.D. & Feinstone, S.M. Epidemiology. In: *Hepatitis A*. Gust, I.D. (ed.). CRC Press, Florida, 1988, pp. 163–191.
7. Lemon, S.M. Type A viral hepatitis. New developments in an old disease. *N Engl J Med* 1985, **313**: 1059–1067.
8. Yang, N.Y., Yu, P.H., Mao, Z.X., Chen, N.L., Chai, S.A. & Mai, J.L. Inapparent infection of hepatitis A virus. *Am J Epidemiol* 1988, **127**: 599–604.
9. Schiff, E.R. Atypical clinical manifestations of hepatitis A. *Vaccine* 1992, **10**: S18–S19.
10. Glikson, M., Galun, E., Oren, R., Tur-Kapsa, R. & Shouval, D. Relapsing hepatitis A: a review of 14 cases and literature survey. *Medicine (Baltimore)* 1992, **71**: 14–23.
11. Gust, I.D. & Feinstone, S.M. Clinical features. In: *Hepatitis A*. Gust, I.D. (ed.). CRC Press, Florida, 1988, pp. 145–162.
12. Gust, I.D. & Feinstone, S.M. General preventive measures. In: *Hepatitis A*. Gust, I.D. (ed.). CRC Press, Florida, 1988, pp. 163–191.
13. Lemon, S.M. Inactivated hepatitis A virus vaccines. *Hepatology* 1992, **15**: 1194–1197.
14. Andre, F.E., D'Hondt, E., Delem, A. & Safary, A. Clinical assessment of the safety and efficacy of an inactivated hepatitis A vaccine: rationale and summary of findings. *Vaccine* 1992, **10**: S160–S169.
15. Fujiyama, S., Iino, S., Odoh, K. *et al.* Time course of hepatitis A virus antibody titre after active and passive immunization. *Hepatology* 1992, **15**: 983–988.
16. Werzberger, A., Mensch, B., Kuter, B. *et al.* A controlled trial of formalin-inactivated hepatitis A vaccine in healthy children. *N Engl J Med* 1992, **327**: 453–457.
17. Thieme, T., Yoshihara, P., Piacentini, S. & Beller, M. Clinical evaluation of oral body fluid samples for diagnosis of viral hepatitis. *J Clin Microbiol* 1992, **30**: 1076–1079.
18. Stuart, J.M., Majeed, F.A., Cartwright, K.A.V. *et al.* Salivary antibody testing in a school outbreak of hepatitis A. *Epidemiol Infect* 1992, **109**: 161–166.
19. Cossar, J.H. & Reid, D. Not all travellers need immunoglobulin for hepatitis A. *Br Med J* 1987, **294**: 1503.
20. Gust, I.D. Design of hepatitis D vaccines. In: Zuckerman, A.J. (ed.) *British Medical Bulletin: Viral Hepatitis*. Churchill Livingstone, London, 1990, pp. 319–328.
21. Flehmig, B., Heinricy, U. & Pfisterer, M. Immunogenicity of a recombinant hepatitis A vaccine in seronegative volunteers. *Lancet* 1989, **i**: 1039.
22. Feinstone, S.M., Daemer, R.J., Gust, I.D. *et al.* Liver attenuated vaccine for hepatitis A. *Dev Biol Stand* 1983, **54**: 429–432.
23. Maynard, J.E., Kane, M.A., Alter, M.J. & Hadler, S.C. Control of hepatitis B by immunization: global perspective. In: Zuckerman, A.J. (ed.) *Viral Hepatitis and Liver Diseases*. Alan R. Liss, New York, 1988, pp. 967.
24. *Bull WHO* 1988, **66**: 443.
25. Michel, M.L. & Tiollais, P. Structure and expression of the hepatitis B virus genome. *Hepatology* 1987, **7**: 61S–63S.
26. Neurath, A.R., Strick, N. & Sproul, P. Search for hepatitis B virus cell receptors reveals binding sites for interleukin 6 on the virus envelope protein. *J Exp Med* 1992, **175**: 461–469.
27. Leenders, W.P.J., Glansbeek, H.J., de Bruin, W.C.C. & Yap, S.H. Binding of the major and large HBsAg to human hepatocytes and liver plasma membranes: putative external and internal receptors for infection and secretion of hepatitis B virus. *Hepatology* 1990, **12**: 141–147.
28. Miller, R.H., Kaneko, S., Chung, C.T., Girones, R. & Purcell, R.H. Compact organisation of the hepatitis B virus genome. *Hepatology* 1989, **9**: 322–327.
29. Vento, S., Hegarty, J.E., Alberti, A. *et al.* T lymphocyte sensitisation to HBcAg and T cell mediated unresponsiveness to HBsAg in hepatitis B related chronic liver disease. *Hepatology* 1985, **5**: 192–197.
30. Mondelli, M., Mieli-Vergiani, G., Alberti, A. *et al.* Specificity of T lymphocyte cytotoxicity to autologous hepatocytes in chronic HBV infection: evidence that T cells are directed against HBcAg expressed on hepatocytes. *J Immunol* 1982, **129**: 2773–2778.
31. Naoumov, N.V., Mondelli, M., Alexander, G.J.M. *et al.* Relationship between expression of hepatitis B virus antigens in isolated hepatocytes and autologous lymphocyte cytotoxicity in patients with chronic hepatitis B virus infection. *Hepatology* 1984, **4**: 63–68.
32. Mondelli, M., Bortoletti, F. & Pontisso, P. Definition of HBV specific target antigens recognised by cytotoxic T cells in acute HHV infection. *Clin Exp Immunol* 1987, **68**: 245–250.
33. Ferrari, C., Penn, A., Giuberti, T. *et al.* Intrahepatic nucleocapsid antigen specific T cells in chronic active hepatitis B. *J Immunol* 1987, **139**: 2050–2058.
34. Ferrari, C., Mondelli, M.Y., Penna, A. *et al.* Functional characterisation of cloned intrahepatic HBV nucleoprotein specific helper T cell lines. *J Immunol* 1987, **139**: 539–544.
35. Ou, J., Laub, O. & Rutter, W.J. Hepatitis B virus gene function: the precore region targets the core antigen to cellular membranes and causes the secretion of the e antigen. *Proc Natl Acad Sci USA* 1986, **83**: 1578–1582.
36. Feitelson, M.A. Products of the 'X' gene in hepatitis B and related viruses. *Hepatology* 1986, **6**: 191–198.
37. Katayama, K., Hayashi, N., Sasaki, Y. *et al.* Detection of hepatitis B virus X gene protein and antibody in type B chronic liver disease. *Gastroenterology* 1989, **97**: 990–998.
38. Feitelson, M.A. & Clayton, M.M. X antigen/antibody markers in hepatitis B virus infections. *Gastroenterology* 1990, **99**: 500–507.
39. Yoffe, B. & Noonan, C.A. Hepatitis B virus: new and evolving issues. *Dig Dis Sci* 1992, **37**: 1–9.
40. Brown, J.L., Carman, W.F. & Thomas, H.C. The clinical significance of molecular variation within the hepatitis B virus genome. *Hepatology* 1992, **15**: 144–148.
41. Carman, W.F., Jacyna, M.R., Karriyannis, S. *et al.* Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989, **ii**: 588–591.
42. Brunetto, M.R., 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.
43. Naoumov, N., Schneider, R., Grotzinger, T. *et al.* Precore mutant hepatitis B virus infection and liver disease. *Gastroenterology* 1992, **102**: 538–543.
44. Fagan, E.A., Davison, F., Trowsbridge, R. *et al.* Detection of HBV-DNA sequences in liver in HBsAg sero-negative patients with liver disease with or without anti-HBc antibodies. *Hepatology* 1989, **9** (Suppl): abstract 26.

Hepatitis B virus

23. Maynard, J.E., Kane, M.A., Alter, M.J. & Hadler, S.C. Control of hepatitis B by immunization: global perspective. In: Zuckerman, A.J. (ed.) *Viral Hepatitis and Liver Diseases*. Alan R. Liss, New York, 1988, pp. 967.
24. *Bull WHO* 1988, **66**: 443.

45. Fernholz, D., Stemler, M., Brunetto, M., Bonino, F. & Will, H. Replicating and virion secreting hepatitis B mutant virus unable to produce preS2 protein. *J Hepatol* 1991, **13**: S102–S104.
46. Trent-Mills, C., Lee, E. & Perrillo, R. Relationship between histology, aminotransferase levels, and viral replication in chronic hepatitis B. *Gastroenterology* 1990, **99**: 519–524.
47. Alexander, G.J.M. Immunology of hepatitis B virus infection. In: Zuckerman, A.J. (ed.) *British Medical Bulletin – Viral Hepatitis*. Churchill Livingstone, London, 1990, pp. 354–367.
48. Mieli-Vergiani, G., Vergiani, D., Portmann, B. *et al.* Lymphocyte cytotoxicity to autologous hepatocyte in HBsAg positive chronic liver disease. *Gut* 1982, **23**: 1029–1036.
49. Hurie, M.B., Mast, E.E. & Davis, J.P. Horizontal transmission of hepatitis B virus infection to United States born children of Hmong refugees. *Pediatrics* 1992, **89**: 269–273.
50. Hsu, H.Y., Chang, M.H., Lee, C.Y., Chen, J.S., Hsu, H.C. & Chen, D.S. Spontaneous loss of HBsAg in children with chronic hepatitis B infection. *Hepatology* 1992, **15**: 382–386.
51. Ghaffar, Y.A., Fattah, S.A., Kamel, M., Badr, R.M., Mahomed, F.K. & Strickland, T. The impact of endemic schistosomiasis on acute viral hepatitis. *Am J Trop Med Hyg* 1991, **45**: 743–750.
52. Ikeda, T., Lever, A.M.L. & Thomas, H.C. Evidence for a deficiency of interferon production in patients with chronic hepatitis B virus acquired in adult life. *Hepatology* 1986, **6**: 962.
53. Zachoval, R., Abb, J., Zachoval, V. *et al.* Interferon alpha in hepatitis B and non-A, non-B defective production by peripheral blood mononuclear cells in chronic infection and development of serum interferon in acute disease. *J Hepatol* 1988, **6**: 364–368.
54. Twu, J.S., Lee, C.H., Lin, M.P. & Schloemer, R.H. Hepatitis B virus suppresses expression of human interferon. *Proc Natl Acad Sci USA* 1988, **85**: 252–256.
55. Onji, M., Lever, A.M.L., Saito, L. & Thomas, H.C. Defective response to interferons in cells transfected with the hepatitis B virus genome. *Hepatology* 1989, **9**: 92–96.
56. De Martino, M., Rossi, M.E.R., Muccioli, A.T., Resti, M. & Vierucci, A. Interference of hepatitis B virus surface antigen with natural kill cell function. *Clin Exp Immunol* 1985, **61**: 90–95.
57. Zhao, W., Egan, M. & Peters, M. B cell responses in patients with acute viral hepatitis B. *Proceedings of the AASLD 1990 Immunology and the Liver Meeting*, pp. 29.
58. Anastassakos, Ch., Alexander, G.J.M., Wolstencroft, R.A. *et al.* Interleukin 1 and interleukin 2 activity in chronic hepatitis B virus infection. *Gastroenterology* 1988, **94**: 999–1005.
59. Sheron, N., Lau, J., Daniels, H.M. *et al.* Increased production of tumour necrosis factor-alpha in chronic hepatitis B virus infection. *J Hepatol* 1991, **12**: 241–245.
60. Alexander, G.J.M., Brahm, J., Fagan, E.A. *et al.* Loss of HBsAg with interferon therapy in chronic hepatitis B virus infection. *Lancet* 1987, **ii**: 66–69.
61. Lok, A.S.F., Novick, D.M., Karayiannis, P. *et al.* A randomised study of the effects of adenine arabinoside 5'-monophosphate (short or long courses) and lymphoblastoid interferon on hepatitis B virus replication. *Hepatology* 1985, **5**: 1132–1138.
62. Dusheiko, G., Dibisceglie, S., Bowyer, S. *et al.* Recombinant leukocyte interferon treatment of chronic hepatitis B. *Hepatology* 1985, **5**: 556–560.
63. Perrillo, R.P., Schiff, E., Davis, G.L. *et al.* A randomised controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. *N Engl J Med* 1990, **323**: 295–301.
64. Hoofnagle, J.H., Peters, M., Mullen, K.D. *et al.* Randomized controlled trial of recombinant human alpha-interferon in patients with chronic hepatitis B. *Gastroenterology* 1988, **95**: 1318–1325.
65. Dooley, J.S., Vergalla, J., Hoofnagle, J.H., Zoon, K.C., Munson, P.J. & Jones, E.A. Specific binding of human alpha interferon to high affinity cell surface binding sites on peripheral blood mononuclear cells. *J Lab Clin Med* 1989, **113**: 623–631.
66. Lau, J.Y.N., Sheron, N., Morris, A.G., Bomford, A.B., Alexander, G.J.M. & Williams, R. Interferon-alpha receptor expression and regulation in chronic hepatitis B virus infection. *Hepatology* 1990, **13**: 332–338.
67. Lisker-Melman, M., Di Bisceglie, A.M., Usala, S.J., Weintraub, B., Murray-Lyon, I.M. & Hoofnagle, J.H. Development of thyroid disease during therapy of chronic viral hepatitis with interferon alpha. *Gastroenterology* 1992, **102**: 2155–2160.
68. Foraciari, G., Bassi, C., Beltrami, M., Castagnetti, E., Maccari, S. & Plancher, A.C. Haemolytic anaemia secondary to interferon treatment for chronic B hepatitis. *J Clin Gastroenterol* 1991, **13**: 596–597.
69. Nevens, F., Goubau, P., Van Eyken, P., Desmyter, J., Desmet, V. & Fevery, J. Treatment of decompensated viral hepatitis B-induced cirrhosis with low doses of interferon alpha. *Liver* 1993, **13**: 15–19.
70. Hoofnagle, J.H., Di Bisceglie, A.M., Waggoner, J.G. & Park, Y. Interferon alpha for patients with clinically apparent cirrhosis due to chronic hepatitis B. *Gastroenterology* 1993, **104**: 1116–1121.
71. Perillo, R., Tamburro, C., Regenstein, F. *et al.* Treatment of decompensated hepatitis B (CHB) with a titratable, low dose regimen of recombinant interferon alpha-2b (rIFNa-2b). *Hepatology* 1992, **16**: 126A (abstract).
72. Santantonio, T., Jung, M.C., Schneider, R., Pastore, G., Pape, G.R. & Will, H. Selection for a Pre-C stop codon mutation in a hepatitis B variant with a pre-c initiation codon mutation during interferon treatment. *J Hepatol* 1991, **13**: 368–371.
73. Doong, S.L., Tsai, C.H., Schinazi, R.F., Liotta, D.C. & Cheng, Y.C. Inhibition of the replication of hepatitis B virus in vitro by 2',3'-dideoxy-3'-thiactidine and related analogues. *Proc Natl Acad Sci USA* 1991, **88**: 8495–8499.
74. Kruskall, M.S., Alper, C.A., Awdeh, Z., Junis, E.J. & Marcus-Bagley, D. The immune response to hepatitis B vaccine in humans: inheritance patterns in families. *J Exp Med* 1992, **175**: 461–469.
75. Brugera, M., Cremades, M., Salinas, R., Costa, J., Grau, M. & Sans, J. Impaired response to recombinant hepatitis B vaccine in HIV-infected persons. *J Clin Gastroenterol* 1992, **14**: 27–30.
76. Zuckerman, A.J. Immunisation against hepatitis B. In: Zuckerman, A.J. (ed.) *British Medical Bulletin: Viral Hepatitis*. Churchill Livingstone, London, 1990, pp. 383–398.
77. Stevens, C.E., Taylor, P.E., Tong, M.J. *et al.* Hepatitis B vaccine: an overview. In: Vyas, G.N., Dienstag, J.L. & Hoofnagle, J.H. (eds) *Viral Hepatitis and Liver Disease*. Grune & Stratton, Orlando, Florida, 1984, p. 275.
78. Catterall, A.P. & Murray-Lyon, I.M. Strategies for hepatitis B immunization. *Gut* 1992, **33**: 576–579.
79. Hatter, S.C., Francis, D.P., Maynard, J.E. *et al.* Long term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. *N Engl J Med* 1986, **315**: 209.
80. Carman, W.F., Zanetti, A.R., Karayiannis, P. *et al.* Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990, **336**: 325–329.
81. Itoh, Y., Takai, E., Ohnuma, H. *et al.* A synthetic peptide vaccine involving the product of the pre-S2 region of hepatitis B virus DNA: protective efficacy in chimpanzees. *Proc Natl Acad Sci USA* 1986, **83**: 9174–9178.

Hepatitis C virus

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

83. Arima, T., Nagashima, H., Murakami, S. *et al.* Cloning of a cDNA associated with acute and chronic hepatitis C infection generated from patients serum RNA. *Gastroenterol Jpn* 1989, **24**: 540–544.
84. Choo, Q.L., Weiner, A.J., Overby, L.R., Kuo, G., Houghton, M. & Bradley, D.W. Hepatitis C virus: the major causative agent of viral non-A, non-B hepatitis. In: Zuckerman, A.J. (ed.) *British Medical Bulletin. Viral Hepatitis*. Churchill Livingstone, London, 1990, pp. 423–441.
85. Alter, H.J., Purcell, R.H., Shih, J.W. *et al.* Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N Engl J Med* 1989, **321**: 1494–1500.
86. Van Der Poel, C.L., Cuypers, H.T.M., Reesink, H.W. *et al.* Confirmation of hepatitis C virus infection by new four-antigen recombinant immunoblot assay. *Lancet* 1991, **337**: 317–319.
87. Katayama, T., Mazda, T., Kikuchi, S. *et al.* Improved serodiagnosis of non-A, non-B hepatitis by an assay detecting antibody to hepatitis C virus core antigen. *Hepatology* 1992, **15**: 391–394.
88. Donahue, J.G., Munoz, A., Ness, P.M. *et al.* The declining risk of post-transfusion hepatitis C virus infection. *N Engl J Med* 1992, **327**: 369–373.
89. Bukh, J., Purcell, R.H. & Miller, R.H. Importance of primer selection for detection of hepatitis C virus with the polymerase chain reaction assay. *Proc Natl Acad Sci USA* 1992, **89**: 187–191.
90. Sugitani, M., Inchauspe, G., Shindo, M. & Prince, A.M. Sensitivity of serological assays to identify blood donors with hepatitis C viraemia. *Lancet* 1992, **339**: 1018–1019.
91. Puoti, M., Zonaro, A., Ravaggi, A., Marin, M.G., Castelnovo, F. & Cariani, E. Hepatitis C virus RNA and antibody response in the clinical course of acute hepatitis C infection. *Hepatology* 1992, **16**: 877–881.
92. Alberti, A., Morsica, G., Chemello, L. *et al.* Hepatitis C viraemia and liver disease in symptom-free individuals with anti-hepatitis C virus. *Lancet* 1992, **340**: 697–698.
93. Machida, A., Ohnuma, H., Tsuda, F. *et al.* Two distinct subtypes of hepatitis C virus defined by antibodies to the putative core region. *Hepatology* 1992, **16**: 886–891.
94. Mitsui, T., Iwano, K., Masuko, K. *et al.* Hepatitis C virus infection in medical personnel after needlestick accident. *Hepatology* 1992, **16**: 1109–1114.
95. Hsu, H.H., Wright, T.L., Luba, D. *et al.* Failure to detect hepatitis C virus genome in human secretions with polymerase chain reaction. *Hepatology* 1991, **14**: 760–767.
96. Fried, M.W., Shindo, M., Fong, T.-L., Fox, P.C., Hoofnagle, J.H. & DiBisceglie, A.M. Absence of hepatitis C RNA from saliva and semen of patients with chronic hepatitis C. *Gastroenterology* 1992, **102**: 1306–1308.
97. Klein, R.S., Freeman, K., Taylor, P. & Stevens, C.E. Occupational risks for hepatitis C virus infection among New York dentists. *Lancet* 1991, **338**: 1539–1542.
98. Brettler, D.B., Mannucci, P.M., Gringeri, A. *et al.* Low risk of hepatitis C virus transmission among sexual partners of hepatitis C virus infected haemophilic males: an international multicentre study. *Blood* 1992, **80**: 540–543.
99. Hollinger, F.B. & Lin, H.J. Community-acquired hepatitis C virus infection. *Gastroenterology* 1992, **102**: 969–972.
100. DiBisceglie, A.M., Goodman, Z.D., Ishak, K.G., Hoofnagle, J.H., Melpolder, J.J. & Alter, H.J. Long-term clinical and histological follow up of chronic post transfusion hepatitis. *Hepatology* 1991, **14**: 969–974.
101. Dittmann, S., Roggendorf, M., Durkop, J., Wiese, M., Lorbeer, B. & Dienhardt, F. Long term persistence of hepatitis C virus antibodies in a single source outbreak. *J Hepatol* 1991, **13**: 323–327.
102. Scheuer, P.J., Ashrafzadeh, P., Sherlock, S., Brown, D. & Dusheiko, G.M. The pathology of hepatitis C. *Hepatology* 1992, **15**: 567–571.
103. Tine, F., Magrin, S., Craxi, A. & Pagliaro, L. Interferon for non-A, non-B hepatitis: a meta analysis of randomized clinical trials. *J Hepatol* 1991, **13**: 192–199.
104. Shindo, M., DiBisceglie, A.M. & Hoofnagle, J.H. Long term follow-up of patients with chronic hepatitis C treated with α -interferon. *Hepatology* 1992, **15**: 1013–1026.
105. DiBisceglie, A.M., Shindo, M., Fong, T.-L. *et al.* A pilot study of ribavirin therapy for chronic hepatitis C. *Hepatology* 1992, **16**: 649–654.
106. Lunel, F., Abauf, N., Franguel, L. *et al.* Liver/kidney microsome antibody type I and hepatitis C virus infection. *Hepatology* 1992, **16**: 630–636.
107. Michel, G., Ritter, A., Gerken, G., Meyer Zum Buschenfelde, K.-H., Decker, R. & Manns, M.P. Anti-GOR and hepatitis C virus in autoimmune liver disease. *Lancet* 1992, **339**: 267–269.
108. Papo, T., Marcellin, P., Bernuau, J., Durand, F., Poinard, T. & Benhamou, J.-P. Autoimmune chronic hepatitis exacerbated by alpha interferon. *Ann Intern Med* 1992, **116**: 51–53.
109. McFarlane, I.G., Smith, H.M., Johnson, P.J., Bray, G., Vergani, D. & Williams, R. Significance of possible anti-HCV antibodies in autoimmune chronic active hepatitis. *Lancet* 1990, **335**: 754–757.
110. Nishiguchi, S., Kuroki, T., Ueda, T. *et al.* Detection of hepatitis C virus antibody in the absence of viral RNA in patients with autoimmune hepatitis. *Ann Intern Med* 1992, **116**: 21–25.
111. Misiani, R., Bellavita, P., Fenili, D. *et al.* Hepatitis C virus infection in patients with mixed essential cryoglobulinaemia. *Ann Intern Med* 1992, **117**: 573–577.
112. Agnello, V., Chung, R.T. & Kaplan, L.M. A role for hepatitis C virus infection in type II cryoglobulinaemia. *N Engl J Med* 1992, **327**: 1490–1495.
113. Haddad, J., Deny, P., Munz-Gotheil, C. *et al.* Lymphocytic sialadenitis of Sjogren's syndrome associated with chronic hepatitis C virus liver disease. *Lancet* 1992, **339**: 321–323.

Hepatitis D and E

114. Monjardino, J.P. & Saldanha, J.A. Delta hepatitis – the disease and the virus. In: Zuckerman, A.J. (ed.) *British Medical Bulletin: Viral Hepatitis*. Churchill Livingstone, London, 1990, pp. 399–407.
115. Hoofnagle, J.H. Type D (delta) hepatitis. *JAMA* 1989, **261**: 1321.
116. Rizzetto, M. & Verme, G. Delta hepatitis. *J Hepatol* 1985, **1**: 187–193.
117. Chu, C.M. & Liaw, Y.F. Studies on the composition of the mononuclear cell infiltrates in liver from patients with chronic active delta hepatitis. *Hepatology* 1989, **10**: 911–915.
118. Lenzi, M., Fusconi, M., Cassani, F. *et al.* Characterisation of LKM1 and LKM2 antigens. *Prog Clin Biol Res* 1987, **234**: 343–345.
119. Ottobrelli, A., Marzano, A., Smedile, A. *et al.* Patterns of HDV reinfection and disease in liver transplantation. *Gastroenterology* 1991, **56**: 1649–1655.
120. Naidu, S.S. & Viewanath, R. Infectious hepatitis in Delhi (1955–6): a critical study; observations in pregnant women. *Indian J Med Res* 1957, **45** (Suppl 1): 71–76.
121. Goldsmith, R., Yarbrough, P.O., Reyes, G. *et al.* Enzyme-linked immunosorbent assay for diagnosis of acute sporadic hepatitis E in Egyptian children. *Lancet* 1992, **339**: 328–331.
122. Centres for Disease Control. Hepatitis E among US travellers, 1989–1992. *MMWR CDC Surveill Summ* 1993, **42**: 1–3.

Metabolic liver disease

123. Simon, M., Bourel, M., Fauchet, R. & Genetet, B. Association of HLA A3 and HLA B14 antigens with idiopathic haemochromatosis. *Gut* 1976, **17**: 332–334.

124. Powell, L.W., Summers, K.M., Board, P.G., Axelsen, P.G., Webb, S. & Halliday, J.W. Expression of haemochromatosis in homozygous subjects: implications for early diagnosis and prevention. *Gastroenterology* 1990, **98**: 1625–1632.
 125. Weber, J.L. & May, P.E. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Ann Hum Genet* 1989, **44**: 388–396.
 126. Eriksson, S. & Larsson, C. Purification and partial characterization of PAS-positive inclusion bodies from the liver in α -1-antitrypsin deficiency. *N Engl J Med* 1975, **292**: 176–180.
 127. Carlson, J.A., Rogers, B.B., Siefers, R.N. *et al.* The accumulation of PiZ α -1-antitrypsin causes liver damage in transgenic mice. *J Clin Invest* 1989, **83**: 1183–1190.
 128. Eriksson, S. α -1-antitrypsin deficiency and liver cirrhosis in adults. *Acta Med Scand* 1987, **221**: 461–467.
 129. Sifers, R.N., Finegold, M.J. & Wood, S.L.C. Molecule biology and genetics of α -1-antitrypsin deficiency. *Semin Liver Dis* 1992, **12**: 414–436.
 130. Lomas, D.A., Evans, D.L.I., Finch, J.T. & Carrell, R.W. The mechanism of Z α -1-antitrypsin accumulation in the liver. *Nature* 1992, **357**: 605–607.
- Portal hypertension**
131. Schrier, R.W., Arroyo, V., Bernardi, M. *et al.* Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988, **8**: 1151–1157.
 132. Colombato, L.A., Albillos, A. & Groszmann, R.J. Temporal relationship of peripheral vasodilatation, plasma volume expansion and the hyperdynamic circulatory state in portal hypertensive rats. *Hepatology* 1991, **15**: 323–328.
 133. Palmer, R.M., Ashton, D.S. & Moncada, S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988, **333**: 664–666.
 134. Stark, M.E. & Szurzewski, J.H. Role of nitric acid in gastrointestinal and hepatic function and disease. *Gastroenterology* 1992, **103**: 1928–1949.
 135. Pizcueta, P., Pique, J.M., Fernandez, M. *et al.* Modulation of the hyperdynamic circulation of cirrhotic rats by nitric oxidase inhibitor. *Gastroenterology* 1992, **103**: 1909–1915.
 136. Pizcueta, V.S., Sogni, P., Moreau, R. *et al.* Evidence for normal nitric oxide: mediated vasodilator tone in conscious rats with cirrhosis. *Hepatology* 1992, **16**: 980–983.
 137. Vallance, P.J.T. & Moncada, S. Hyperdynamic circulation in cirrhosis: a role for nitric oxide. *Lancet* 1991, **337**: 776–778.
 138. Kleber, G., Sauerrusch, T., Ansari, H. & Paumgartner, G. Prediction of variceal haemorrhage in cirrhosis: a prospective follow-up study. *Gastroenterology* 1991, **100**: 1332–1337.
 139. Rigo, G.P., Merighi, A., Chahin, M.J. *et al.* A prospective study of the ability of three endoscopic classifications to predict haemorrhage from esophageal varices. *Gastrointest Endosc* 1992, **38**: 425–429.
 140. The North Italian Endoscopic Club for the Study and Treatment of Esophageal Varices. Prediction of the first variceal haemorrhage in patients with cirrhosis of the liver and esophageal varices; a prospective multicentre study. *N Engl J Med* 1988, **319**: 983–989.
 141. Merkel, C., Bolognesi, M., Bellon, Z. *et al.* Prognostic usefulness of hepatic vein catheterization in patients with cirrhosis and esophageal varices. *Gastroenterology* 1992, **102**: 973–975.
 142. Pagliaro, L., Burroughs, A.K., Sorensen, T.I.A. *et al.* Therapeutic controversies and randomised controlled trials (RCTs): prevention of bleeding and rebleeding in cirrhosis. *Gastroenterol Int* 1989, **2**: 71–84.
 143. Pagliaro, L., D'Amico, G., Sorensen, T.I. *et al.* Prevention of first bleeding in cirrhosis: a meta-analysis of randomized trials of nonsurgical treatment. *Ann Intern Med* 1992, **117**: 59–70.
 144. Van Ruiswyk, J. & Byrd, J.C. Efficacy of prophylactic sclerotherapy for prevention of a first variceal haemorrhage. *Gastroenterology* 1992, **102**: 587–597.
 145. Garcia-Pagan, J.C., Feu, F., Bosch, J. & Rodes, J. Propranolol compared with propranolol plus isosorbide-5-mononitrate for portal hypertension in cirrhosis: a randomized controlled study. *Ann Intern Med* 1991, **114**: 869–873.
 146. Stiegmann, G.V., Goff, J.S., Michaelitz-Onody, P.A. *et al.* Endoscopic sclerotherapy as compared with endoscopic ligation for bleeding oesophageal varices. *N Engl J Med* 1992, **326**: 1527–1532.
 147. Gimson, A.E.S., Ramage, J.K., Panos, M.Z. *et al.* Randomized trial of variceal banding ligation versus injection sclerotherapy for bleeding oesophageal varices. *Lancet* 1993, **342**: 391–394.
 148. MacMathuna, P., Westaby, D. & Williams, R. Taking the tension out of the portal system: an approach to the management of portal hypertension in the 1990's. *Scand J Gastroenterol* 1990, **25** (Suppl 175): 131–145.
 149. Walker, S., Kreichgauer, H.P. & Bode, J.C. Terlipressin vs. somatostatin in bleeding oesophageal varices: a controlled, double blind study. *Hepatology* 1992, **15**: 1023–1030.
 150. Shields, R., Jenkins, S.A., Baxter, J.N. *et al.* A prospective randomized trial comparing the efficacy of somatostatin with injection sclerotherapy in the control of bleeding oesophageal varices. *J Hepatol* 1992, **15**: 128–137.
 151. Gimson, A.E.S., Westaby, D. & Williams, R. Endoscopic sclerotherapy and management of gastric variceal haemorrhage. *J Hepatol* 1991, **13**: 274–278.
 152. Viavianos, P., Gimson, A.E., Westaby, D. & Williams, R. Balloon tamponade in variceal bleeding: use and misuse. *Br Med J* 1989, **298**: 1158.
 153. Panes, J., Bordas, J., Pique, J. *et al.* Increased gastric mucosal perfusion in cirrhotic patients with portal hypertensive gastropathy. *Gastroenterology* 1992, **103**: 1875–1882.
 154. Tanoue, K., Hashizume, M., Wada, H. *et al.* Effects of endoscopic injection sclerotherapy on portal hypertensive gastropathy: a prospective study. *Gastrointest Endosc* 1992, **38**: 582–585.
 155. Wang, T.F., Lee, F.Y., Tsai, Y.T. *et al.* Relationship of portal pressure, anorectal varices and haemorrhoids in cirrhotic patients. *J Hepatol* 1992, **15**: 170–173.
 156. Naveau, S., Bedossa, P., Poynard, T., Mory, B. & Chaput, J.C. Portal hypertensive colopathy: a new entity. *Dig Dis Sci* 1991, **36**: 1774–1781.
- Transcutaneous intra-hepatic portosystemic shunts**
157. Rosch, J., Antonovic, R. & Dotter, C.T. Transjugular approach to the liver, biliary system and portal circulation. *Am J Roentgenol Radium Ther Nucl Med* 1975, **125**: 602–608.
 158. Vinel, J.P., Scotto, J.M., Levade, M. *et al.* Embolisation des varices oesophagienne par voie transjugulaire dans les hemorrhagies digestives graves du cirrhotique. Etude prospective de 83 patients. *Gastroenterol Clin Biol* 1985, **9**: 814–818.
 159. Colapinto, R.F., Stonell, R.D., Birch, S.J. *et al.* Creation of an intrahepatic portosystemic shunt with a Gruntzig balloon catheter. *Can Med Assoc J* 1982, **126**: 267–268.
 160. Colapinto, R.F., Stronell, D., Gildner, M. *et al.* Formation of intrahepatic portosystemic shunts using a balloon dilation catheter: preliminary clinical experience. *Am J Roentgenol* 1983, **140**: 709–714.
 161. Rossle, M., Richter, G.M., Noldge, G. *et al.* Performance of an intrahepatic portocaval shunt (PCS) using a catheter technique: a case report. *Hepatology* 1988, **8**: 1348.
 162. Ring, E.J., Lake, J.R., Roberts, J.P. *et al.* Using transjugular intrahepatic portosystemic shunts to control variceal bleeding before liver transplantation. *Ann Intern Med* 1992, **116**: 304–309.

163. Zemel, G., Becker, G.J., Bancroft, J.W., Benenati, J.F. & Katzen, B.T. Technical advances in transjugular intrahepatic portosystemic shunts. *Radiographics* 1992, **12**: 615–622.
 164. Rossle, M., Noldge, G., Perarnau, J.M. *et al.* Transjugular intrahepatic portosystemic stent-shunt (TIPS): experience with an improved technique. *Hepatology* 1991, **14**: 96.
 165. Perarnau, J.M., Noldge, G. & Rossle, M. Intrahepatic portacaval anastomosis by the transjugular approach. Use of the Palmaz endoprosthesis. *Presse Med* 1991, **20**: 1770–1772.
 166. Rossle, M. & Haag, K. Interventional treatment of portal hypertension. *Dig Dis* 1992, **10** (Suppl 1): 94–102.
 167. LaBerge, J.M., Ring, E.J., Lake, J.R. *et al.* Transjugular intrahepatic portosystemic shunts: preliminary results in 25 patients. *J Vasc Surg* 1992, **16**: 258–267.
 168. Haag, K. & Rossle, M. Transjugular intrahepatic portosystemic stent-shunt (TIPS): a new technique in the treatment of portal hypertension. In: Kurathong, S. (ed.) *Update on Gastrointestinal and Hepato-Biliary Disorders*. Unity Publication, Bangkok, 1992, pp. 53–66.
 169. Rossle, M., Richter, G.M., Noldge, G. *et al.* Transjugular intrahepatic portosystemic stent shunt: a two year follow-up. *Dig Dis* 1992, **9**: 6–12.
 170. Ochs, A., Sellinger, M., Haag, K. *et al.* Transjugular intrahepatic portosystemic stent-shunt for refractory ascites and hepatorenal syndrome: results of a pilot study. *Gastroenterology* 1992, **102**: 863.
 171. Sellinger, M., Ochs, A., Haag, K., Noldge, G., Gerok, W. & Rossle, M. Incidence of hepatic encephalopathy and follow-up of liver function in patients with transjugular intrahepatic portosystemic shunt (TIPS). *Gastroenterology* 1992, **102**: 883.
- Fulminant hepatic failure**
172. Trey, C. & Davidson, C.S. *The Management of Fulminant Hepatic Failure*. Grune & Stratton, New York, 1970, pp. 282–298.
 173. Prescott, L.F., Illingworth, R.N., Critchley, J.A.J.H., Stewart, M.J., Adam, R.D. & Proudfoot, A.T. Intravenous N-acetylcysteine: the treatment of choice for paracetamol poisoning. *Br Med J* 1979, **2**: 1097–1100.
 174. Harrison, P., Keays, R., Bray, G., Alexander, G. & Williams, R. Improved outcome in paracetamol-induced fulminant hepatic failure following late administration of N-acetylcysteine. *Lancet* 1990, **335**: 1572–1573.
 175. Harrison, P., Wendon, J.A., Grimson, A.E.S., Alexander, G.J.M. & Williams, R. Improvement by acetylcysteine of systemic hemodynamics and oxygen transport in fulminant hepatic failure. *N Engl J Med* 1991 (in press).
- Liver transplantation**
176. Sorrell, M.F., Dovavan, J.P. & Shaw, B.W. Transplantation in the alcoholic: a stalking horse for a larger problem (Editorial). *Gastroenterology* 1992, **102**: 1806–1808.
 177. McMurray, K.R., Baliga, P., Merion, R.M. *et al.* Resource utilization and outcome of liver transplantation for alcoholic cirrhosis. *Arch Surg* 1992, **127**: 772–777.
 178. Lucey, M.R., Merion, R.M., Henley, K.S. *et al.* Selection for and outcome of liver transplantation in alcoholic liver disease. *Gastroenterology* 1992, **102**: 1736–1741.
 179. Pontisso, P., Poon, M.C., Tiollais, P. & Brechot, C. Detection of hepatitis B virus DNA in mononuclear blood cells. *Br Med J* 1984, **288**: 1563–1566.
 180. Davison, F., Alexander, G.J.M., Trowbridge, R., Fagan, E.A. & Williams, R. Detection of hepatitis B virus DNA in spermatozoa, urine, saliva and leukocytes, of chronic HBsAg carriers: a lack of relationship with serum markers of replication. *J Hepatol* 1987, **4**: 37–44.
 181. O'Grady, J.G., Smith, H.M., Davis, S.E. *et al.* Hepatitis B virus reinfection after orthotopic liver transplantation: serological and clinical implications. *J Hepatol* 1992, **14**: 104–111.
 182. Davies, S.E., Portmann, B., O'Grady, J.G. *et al.* Hepatic histology following transplantation for chronic hepatitis B virus infection including a unique pattern of fibrosing cholestatic hepatitis. *Hepatology* 1991, **13**: 150–157.
 183. Lauchart, W., Muller, R. & Pichlmayr, R. Immunoprophylaxis of hepatitis B virus reinfection in recipients of human allografts. *Transplant Proc* 1987, **19**: 2387–2389.
 184. Martin, P., Munoz, S.J. & Friedman, L.S. Liver transplantation for viral hepatitis: current status. *Am J Gastroenterol* 1992, **87**: 409–418.
 185. Reynes, M., Zigneg, L., Samuel, D. *et al.* Graft hepatitis delta virus reinfection after orthotopic liver transplantation in HDV cirrhosis. *Transplant Proc* 1989, **21**: 2424–2425.
 186. McMahon, G., Ehrlich, P.H., Moustafa, Z.A. *et al.* Genetic alterations in the gene encoding the major HBsAg: DNA and immunological analysis of recurrent HBsAg derived from monoclonal antibody-treated liver transplant patients. *Hepatology* 1992, **103**: 323–329.
 187. Shah, G., Demetris, A.J., Gavaler, J.S. *et al.* Incidence, prevalence, and clinical course of hepatitis C following liver transplantation. *Gastroenterology* 1992, **103**: 323–329.
 188. Poterucha, J.J., Rakela, J., Lumeng, L. *et al.* Diagnosis of chronic hepatitis C after liver transplantation by the detection of viral sequences with polymerase chain reaction. *Hepatology* 1992, **15**: 42–45.
 189. Rakela, J. Hepatitis C viral infection in live transplant patients. How bad is it really? *Gastroenterology* 1992, **103**: 338–339.
 190. Pereira, B.J.G., Milford, E.L., Kirkman, R.L. *et al.* Prevalence of hepatitis C virus RNA in organ donors positive for hepatitis C antibody and in the recipients of their organs. *N Engl J Med* 1992, **327**: 910–915.
 191. Wright, T.L., Donegan, E., Hsu, H.H. *et al.* Recurrent and acquired hepatitis C viral infection in liver transplant recipients. *Gastroenterology* 1992, **103**: 317–322.
 192. Ferrell, L.D., Wright, T.L., Roberts, J., Ascher, N. & Lake, J. Hepatitis C viral infection in liver transplant recipients. *Hepatology* 1992, **16**: 865–876.
 193. Roth, D., Fernandez, J.A., Babishkin, S. *et al.* Detection of hepatitis C virus infection among cadaver organ donors: evidence for low transmission of disease. *Ann Intern Med* 1992, **117**: 470–475.
 194. Konig, V., Bauditz, J., Lobeck, H. *et al.* Hepatitis C virus reinfection in allografts after orthotopic liver transplantation. *Hepatology* 1992, **16**: 1137–1143.
 195. McPeake, J.R., O'Grady, J.G., Zaman, S. *et al.* Liver transplantation for hepatocellular carcinoma: tumour size and number determines outcome. *J Hepatol* 1993, **18**: 226–234.
 196. Yokoyama, I., Sheahan, D.G., Carr, B. *et al.* Clinicopathological factors affecting patients survival and tumour recurrence after orthotopic liver transplantation for hepatocellular carcinoma. *Transplantation Proc* 1991, **23**: 2194–2196.
 197. Gores, G.J. & Steers, J.L. Progress in orthotopic liver transplantation for hepatocellular carcinoma. *Gastroenterology* 1993, **104**: 317–320.
 198. Bismuth, H., Morino, M., Sherlock, D. *et al.* Primary treatment for hepatocellular carcinoma by arterial chemoembolization. *Am J Surg* 1992, **163**: 387–394.
 199. Stone, M.J., Klintmalm, C.B.G., Polter, D. *et al.* Neoadjuvant chemotherapy and liver transplantation for hepatocellular carcinoma: a pilot study in 20 patients. *Gastroenterology* 1993, **104**: 196–202.
 200. Carr, B.I., Selby, R., Madariaga, J., Iwatsuki, S. & Starzl, T.E. Prolonged survival after liver transplantation and cancer chemotherapy for advanced-stage hepatocellular carcinoma. *Transplantation Proc* 1993, **25**: 1128–1129.
 201. Rizzi, P.M., Ryder, S.D., Ramage, J.K. *et al.* Neoadjuvant chemotherapy after liver transplantation for hepatocellular carcinoma. (Submitted for publication).

202. O'Grady, J.G., Polson, R.J., Rolles, K., Calne, R.Y. & Williams, R. Liver transplantation in malignant disease: results in 93 consecutive patients. *Ann Surg* 1988, **207**: 373–379.
 203. Dickson, E.R., Murtaugh, P.A., Wiesner, R.H. *et al.* Primary sclerosing cholangitis: refinement and validation of survival models. *Gastroenterology* 1992, **103**: 1893–1901.
 204. Sanchez-Urdazpal, L., Gores, G.J., Ward, E.M. *et al.* Ischaemic-type biliary complications after orthotopic liver transplantation. *Hepatology* 1992, **16**: 49–53.
 205. Sanchez-Urdazpal, L., Gores, G.J., Ward, E.M. *et al.* Diagnostic features and clinical outcome of ischaemic biliary complications after liver transplantation. *Hepatology* 1993, **17**: 554–559.
 206. Wolfsen, H.C., Porayko, M.K., Hughes, R.H. *et al.* The role of endoscopic retrograde cholangiopancreatography after orthotopic liver transplantation. *Am J Gastroenterol* 1992, **87**: 955–960.
 207. Calne, R.Y., Rolles, K., White, D.J.G. *et al.* Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreas, 2 liver. *Lancet* 1979, **ii**: 1033–1036.
 208. Kino, T., Hatanaka, H., Miyata, S. *et al.* FK506, a novel immunosuppressant isolated from streptomyces. Immunosuppressive effects of FK506 in vitro. *J Antibiot (Tokyo)* 1987, **40**: 1256–1265.
 209. Demetris, A.J., Fung, J.J., Todo, S. *et al.* Conversion of liver allograft recipients from cyclosporine to FK506 immunosuppressive therapy – a clinicopathologic study of 96 patients. *Transplantation* 1992, **53**: 1056–1062.
 210. Fung, J., Abu-Elmagid, K., Jain, A. *et al.* A randomized trial of primary liver transplantation under immunosuppression from FK506 vs. cyclosporine. *Transplant Proc* 1991, **23**: 2977–2983.
 211. Markus, B.H., Duquesnoy, R.J., Gordon, R.D. *et al.* Histocompatibility in liver transplant outcome. Does HLA exert a dualistic effect? *Transplantation* 1988, **46**: 372–377.
 212. Yagihashi, A., Kobayashi, M., Noguchi, K. *et al.* HLA matching effect in liver transplantation. *Transplant Proc* 1992, **24**: 2432–2433.
 213. O'Grady, J.G., Alexander, G.J.M., Sutherland, S. *et al.* Cytomegalovirus infection and donor/recipient HLA antigens: interdependent co-factors in pathogenesis of vanishing bile duct syndrome after liver transplantation. *Lancet* 1988, **ii**: 302–305.
 214. Davies, H.S., Pollard, S.G. & Calne, R.Y. Tolerogenic and immunosuppressive properties of liver grafts in animals and man. *Immunol Lett* 1991, **29**: 69–72.
 215. Muller-Rucholty, W. Specific down-regulation of allograft reactivity at the cellular level: graft cells and responder T cells. *Immunol Lett* 1992, **32**: 1–6.
 216. Sachs, D.H. Specific transplantation tolerance. *N Engl J Med* 1991, **325**: 1240–1241.
 217. Starzl, T.E., Demetris, A.J., Trucco, M. *et al.* Chimerism after liver transplantation for type IV glycogen storage disease and type I Gaucher's disease. *N Engl J Med* 1993, **328**: 745–749.
 218. Starzl, T.E., Demetris, A.J., Murase, N. *et al.* Cell migration, chimerism and graft acceptance. *Lancet* 1992, **339**: 1579–1582.
 219. Starzl, T.E., Trucco, M., Zeevi, A. *et al.* Mixed chimerism after transplantation: mechanism or marker of specific tolerance. *Hepatology* 1993, **17**: 943–945.
 220. Selby, R., Starzl, T.E., Yunis, E. *et al.* Liver transplantation for Type IV glycogen storage disease. *N Engl J Med* 1991, **324**: 39–42.
 221. Russell, P.S. Some immunological considerations in liver transplantation. *Hepatology* 1984, **4** (Suppl): 76S–78S.
 222. Iltadt, S.T., Wren, S.M., Bluestone, J.A. *et al.* Characterization of mixed allogenic chimeras: immunocompetence, in vitro reactivity and genetic specificity of tolerance. *J Exp Med* 1985, **162**: 231–244.
- Hepatocellular carcinoma**
223. Calvet, X., Bruix, J. & Gines, P. Prognostic features of hepatocellular carcinoma in the West: a multivariate analysis in 206 patients. *Hepatology* 1990, **12**: 753–760.
 224. Okuda, K., Ohtsuki, T., Obata, H. *et al.* Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. *Cancer* 1985, **56**: 918–928.
 225. Bengmark, S., Borjesson, L. & Hafstrom, L. The natural history of primary carcinoma of the liver. *Scand J Gastroenterol* 1971, **6**: 351–355.
 226. Barbara, L., Benzi, G., Gaiani, S. *et al.* Natural history of untreated hepatocellular carcinoma in cirrhosis: a multivariate analysis of prognostic factors of tumour growth rate and patient survival. *Hepatology* 1992, **16**: 132–137.
 227. Bismuth, H., Houssin, D., Ornowski, J. & Meriggi, F. Liver resection in cirrhotic patients: a Western experience. *World J Surg* 1986, **10**: 311–317.
 228. Sciarino, E., Simonetti, R.G., Lemoli, S. & Pagliaro, L. Adriamycin treatment for hepatocellular carcinoma. Experience with 109 patients. *Cancer* 1985, **56**: 251–257.
 229. Chlebowski, R.T., Brzezucha-Adjukiewicz, A., Cowden, A., Block, J.B., Tong, M.J. & Chan, K.K. Doxorubicin (75 mg/m²) for hepatocellular carcinoma: clinical and pharmacokinetic results. *Cancer Treat Rep* 1984, **68**: 487–492.
 230. Lai, C.L., Wu, P.C., Chan, G.C., Lok, A.S. & Lin, H.J. Doxorubicin versus no antitumour therapy in inoperable hepatocellular carcinoma. *Cancer* 1988, **62**: 479–483.
 231. Ohishi, H., Uchida, H., Yoshimura, H. *et al.* Hepatocellular carcinoma detected by iodized oil. Use of anticancer agents. *Radiology* 1985, **154**: 25–29.
 232. Nakakuma, K., Tashiro, S., Uemura, K. *et al.* Studies on anticancer treatment with an oily anticancer drug injected into the ligated hepatic artery for liver cancer (preliminary report). *Nichidoku Iho* 1979, **24**: 675–682.
 233. Takayashu, K., Shima, Y., Muramatsu, Y. *et al.* Hepatocellular carcinoma: treatment with intraarterial iodized oil with and without chemotherapeutic agents. *Radiology* 1987, **162**: 345–351.
 234. Kanematsu, T., Furuta, T., Takenaka, K. *et al.* A 5 year experience of lipiodolization: selective regional chemotherapy for 200 patients with hepatocellular carcinoma. *Hepatology* 1989, **10**: 98–102.
 235. Kasugai, H., Kojima, J., Tatsuta, M. *et al.* Treatment of hepatocellular carcinoma by transcatheter arterial embolization combined with intraarterial infusion of a mixture of cisplatin and ethiodized oil. *Gastroenterology* 1989, **97**: 965–971.
 236. Livraghi, T., Festi, D., Monti, F. & Vettori, C. US-guided percutaneous injection of small hepatic and abdominal tumours. *Radiology* 1986, **161**: 309–312.
 237. Giovannini, M. & Seitz, J.-F. Alcoolisation echoguidee percutanee des tumeurs hepatiques. *Gastroenterol Clin Biol* 1992, **16**: 126–130.
 238. Livraghi, T. Percutaneous ethanol injection of hepatocellular carcinoma: survival after 3 years in 70 patients. *Ital J Gastroenterol* 1992, **24**: 72–74.
 239. Shinya, S., Tagawa, K., Unuma, T. *et al.* Percutaneous ethanol injection therapy: analysis of 77 patients of hepatocellular carcinoma. *Am J Radiol* 1990, **155**: 1221–1226.
 240. Giovanni, M., Seitz, J.F., Rosello, R. & Gauthier, A. Traitement des petits tumeurs hepatiques par injection percutanee echoguidee d'alcool absolu. Resultats chez 8 patients. *Gastroenterol Clin Biol* 1989, **13**: 974–977.
 241. Livraghi, T., Bolondi, L., Lazzaroni, S. *et al.* Percutaneous ethanol injection in the treatment of hepatocellular carcinoma in cirrhosis. A study on 207 patients. *Cancer* 1992, **69**: 925–929.

242. Marin, G., Meier, E., Cesari, A. & Abrahamson, C. Echoguided percutaneous ethanol injection therapy in 35 hepatocellular carcinomas: 3 years experience (abstract). *Proceedings of the World Congress of Gastroenterology, Sydney, 1990*, pp. 1289.
243. Cedrone, A., Rapaccini, G.L., Pompili, M., Grattagliano, A., Aliotta, A. & Trombino, C. Neoplastic seeding complicating percutaneous ethanol injection for treatment of hepatocellular carcinoma. *Radiology* 1992, **183**: 787–788.
244. Koda, M., Okamoto, K., Miyoshi, Y. & Kawasaki, H. Hepatic vascular and bile duct injury after ethanol injection therapy for hepatocellular carcinoma. *Gastrointest Radiol* 1992, **17**: 167–169.
245. Christopherson, W.M., Mays, E.T. & Barrows, G.H. Liver tumours in women on contraceptive steroids. *Obstet Gynecol* 1975, **46**: 221–223.
246. Mays, E.T. & Christopherson, W.M. Hepatic tumours induced by sex steroids. *Sem Liver Dis* 1984, **4**: 147–157.
247. Ohnishi, S., Murakami, T., Moriyama, T., Mitamura, K. & Imawari, M. Androgen and oestrogen receptors in hepatocellular carcinoma and in the surrounding non-cancerous liver. *Hepatology* 1986, **6**: 440–443.
248. Francavilla, A., Polimeno, L., Di Leo, A. *et al.* The effect of tamoxifen on hepatocyte proliferation *in vivo* and *in vitro*. *Hepatology* 1989, **9**: 614–620.
249. Paliard, P., Clement, G., Saez, S., Chabal, J. & Partensky, C. Traitement du carcinome hepatocellulaire par le tamoxifene. *Gastroenterol Clin Biol* 1984, **8**: 680–681.
250. Trinchet, J., Roudil, F., Vaysse, J. & Beaugrand, M. Effects d'une association Tamoxifene Norethisterone chez 16 malades atteints du cacinome hepatocellulaire. *Gastroenterol Clin Biol* 1985, **9**: 455–456.
251. Melia, W.M., Johnson, P.J. & Williams, R. Controlled clinical trial of doxorubicin and tamoxifen versus doxorubicin alone in hepatocellular carcinoma. *Cancer Treat Rep* 1987, **71**: 1213–1216.
252. Farinati, F., De Maria, N., Fornasiero, M. *et al.* Prospective controlled trial with antioestrogen drug tamoxifen in patients with unresectable hepatocellular carcinoma. *Dig Dis Sci* 1992, **37**: 659–662.
253. Lane, D.P. A death in the life of p53. *Nature* 1993, **362**: 786–787.
254. Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C.C. p53 mutations in human cancers. *Science* 1991, **253**: 49–53.
255. Fearon, E.R. & Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell* 1990, **61**: 759–767.
256. Chen, P.-L., Chen, Y., Bookstein, R. & Lee, W.-H. Genetic mechanisms of tumour suppression by the human p53 gene. *Science* 1990, **250**: 1576–1580.
257. Beasley, R.P. & Hwang, L.Y. Hepatocellular carcinoma and hepatitis B virus. *Sem Liver Dis* 1984, **2**: 113–121.
258. Colombo, M.G., Kuo, G., Choo, Q.L. *et al.* Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989, **ii**: 1006–1008.
259. Bruix, J., Barrera, J.M., Calvert, X. *et al.* Anti-hepatitis C virus antibodies in hepatocellular carcinoma and liver cirrhosis in Spain. *Lancet* 1989, **ii**: 1004–1006.
260. Nagasue, N., Yukaya, H., Hamada, T., Hirose, S., Kanashima, R. & Inokuchi, K. The natural history of hepatocellular carcinoma. A study of 100 untreated patients. *Cancer* 1984, **54**: 1461–1465.
261. Wogan, G.N. Aflatoxins as risk factors for hepatocellular carcinoma in humans. *Cancer Res (Suppl)* 1992, **52**: 2114S–2118S.
262. Bressac, B., Kew, M., Wands, J. & Ozturk, M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from Southern Africa. *Nature* 1991, **350**: 429–431.
263. Ozturk, M., Bressac, B. & Puisieux, A. p53 mutations in hepatocellular carcinoma after aflatoxin exposure. *Lancet* 1991, **338**: 1356–1359.
264. Kress, S., Jahn, U.-R., Buchmann, A., Bannasch, P. & Schwartz, M. p53 mutations in human hepatocellular carcinomas from Germany. *Cancer Res* 1992, **52**: 3220–3223.
265. Fujimoto, Y., Hampton, L.L., Luo, L., Wirth, P. & Thorgerisson, S.S. Low frequency of p53 gene mutations in tumours induced by aflatoxin in non-human primates. *Cancer Res* 1992, **52**: 1044–1046.
266. Scorsone, K.A., Zhou, Y.-Z., Butel, J.S. & Slag, B.L. p53 mutations cluster at codon 249 in hepatitis B virus-positive hepatocellular carcinomas from China. *Cancer Res* 1992, **52**: 1635–1638.
267. Hsia, C.C., Kleiner, D.E., Axiotis, C.A. *et al.* Mutations of p53 gene in hepatocellular carcinoma: roles of hepatitis B virus and aflatoxin contamination in the diet. *J Natl Cancer Inst* 1992, **84**: 1638–1641.
268. Ryder, S.D., Pirerra, L.M.M.B., Luo, D. *et al.* p53 mutations in hepatocellular carcinoma: related to geographical area but not viral aetiology. European Association for the Study of the Liver, Barcelona 1993, A211.