Reviews in Medicine

Liver disease

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Introduction

In the last year there have been many significant developments in clinical hepatology covering the fields of vital 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 hepatotrophic 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 systemic involvement with the infection. We have therefore confined our review to primary hepatotrophic 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. 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. HAV still represents a major health problem, particularly in the developing world where sanitation is poor and mass epidemics still occur. In Western populations mass outbreaks are rare but HAV infection is increasingly prevalent among high-risk groups, such as homosexual men. 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. 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. 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.

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. Prolonged cholestasis and the occurrence of a relapsing course are well described but rare in HAV infection. Minor coagulation defects and depressed fibrinogen levels are, in contrast, relatively frequent but have little clinical significance.

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 immunoglobulin preparations. These are well known to be effective in both pre-exposure and post-exposure prophylaxis against HAV infection 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-

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Phylactic therapy for high-risk groups. 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. The current recommendation is that three doses are given over 6 months and following this high levels of antibody can be demonstrated. 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.

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. 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 risk (Table 1). 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.

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 worldwide and the World Health Organization estimates that 40% of them will eventually die of chronic liver disease and/or hepatocellular carcinoma (HCC). 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. Cytokines or their receptors appear to be important in the replication of HBV, 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. Both HbcAg (hepatitis B core antigen) and HBeAg (hepatitis B e antigen) are encoded by the C gene, which includes a pre-C region. Existing evidence suggests that HbcAg is the major determinant responsible for immunogenicity. 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. 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.

There are an increasing number of different mutant HBVs recognized, 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>
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<tr>
<th>Table 1</th>
<th>Groups recommended to have antibody screening for HAV infection and immunization (US guidelines)</th>
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<tr>
<td>Child care centre workers</td>
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<tr>
<td>Institutionalized patients</td>
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<tr>
<td>Medical personnel</td>
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<tr>
<td>Travellers to endemic areas</td>
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<tr>
<td>Contacts of cases</td>
<td></td>
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<tr>
<td>Food handlers</td>
<td></td>
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<tr>
<td>Prison inmates</td>
<td></td>
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<tr>
<td>Military personnel</td>
<td></td>
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<tr>
<td>Homosexual men</td>
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</table>

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. 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. 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. The pre-core mutants have been associated with fulminant hepatitis in Japan and Israel 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 described 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.

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. 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. 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.

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. 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.

Inborn or acquired immune defects, such as other chronic infections 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. 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. Many other immune defects including abnormal natural killer cell function, decreased in vitro anti-HBs production and increased production of interleukin 1 and tumour necrosis factor-alpha have been demonstrated in chronic HBV infection 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. 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 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 dysfunction and, recently, haemolysis 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 appears high. 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. 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. 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. Many combinations of agents and new agents are currently under trial for
patients who do not respond to interferon alpha therapy, the deoxynucleoside agents 5ddC and 5-FSdcdc have been found to be highly active against the virus in vitro 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. Vaccine responsiveness is also impaired in immunocompromised individuals, HIV seropositive patients had a seroconversion rate of less than 25% overall with no subject with a T4 lymphocyte count below 700 cells per microlitre seroconverting after immunization.

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

<table>
<thead>
<tr>
<th>Likely to respond</th>
<th>Unlikely to respond</th>
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<tr>
<td>Females</td>
<td>Males</td>
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<tr>
<td>Infection acquired in adulthood</td>
<td>Infection acquired in childhood</td>
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<tr>
<td>Low pretreatment HBV-DNA</td>
<td>High pretreatment HBV-DNA</td>
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<tr>
<td>High pretreatment transaminases</td>
<td>Low pretreatment transaminases</td>
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<tr>
<td>Chronic active hepatitis on biopsy</td>
<td>Minimal inflammation on biopsy</td>
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<tr>
<td>Heterosexual</td>
<td>Homosexual</td>
</tr>
<tr>
<td>Delta virus negative</td>
<td>Delta virus positive</td>
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<tr>
<td>HIV negative</td>
<td>HIV positive</td>
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Major immunization programmes have been established throughout the world, usually targeting medical personnel and babies born to HBsAg seropositive mothers in most developed countries. 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. 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. Although most of these new infections were subclinical seroconversions, one patient developed clinical hepatitis. HBV mutants may be responsible for some of the patients who developed HBV infection after successful vaccination 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.

**Table III** Response to HBV vaccination: host factors

<table>
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<tr>
<th>Factors predictive of poor HBV vaccine response</th>
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<tr>
<td>Age</td>
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<tr>
<td>Obesity</td>
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<tr>
<td>HIV infection</td>
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<tr>
<td>Immunosuppressive therapy/diseases</td>
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<tr>
<td>Alcohol excess</td>
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<tr>
<td>Chronic disease</td>
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**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.\(^ {89}\) 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-C22\(^ {86}\) (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 detection\(^ {86}\) and is positive within a very short period following infection.\(^ {89}\) PCR positivity is very closely correlated with histological evidence of chronic hepatitis\(^ {92}\) 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.

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

**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
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. 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, it 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. 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. 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. 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. It has proved impossible to detect HCV-RNA in patients with type 1 CAH who have positive first or second generation antibody tests.

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 cases and the cryoprecipitates in this condition are composed of HCV virions and antigen–antibody complexes. Patients with Sjogren's syndrome have a high incidence of HCV infection 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. HDV is able to replicate independently of HBV. HDV probably causes liver damage by a direct cytopathic action 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. Recent studies in transplanted patients have demonstrated subclinical infection with HDV in the absence of replicating HBV.

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. Enzyme-linked immunosorbant 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. At present, there is no vaccine available and commercially available immunoglobulins do not provide immunity 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 described 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. A large array of microsatellite markers have now been described 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 protease 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. 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. 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. The biological basis of the relationship of temperature and liver damage was explained fully in a recent study 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 accumulation. 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 vasodilatation with the primary abnormality occurring in the non-splanchnic circulatory bed which leads to plasma volume expansion and an increase in cardiac index. 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. 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. It has been demonstrated in animal models that antagonists of nitric acid synthetase can reverse the haemodynamic parameters seen in portal hypertension, although the underlying pathophysiology and mechanism of this response is disputed. 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. 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. 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.

Beta-blockers have been shown to reduce the incidence of the first bleed but do not improve outcome in most trials as the dominant influence on survival is liver function. The lack of any survival advantage in many studies has resulted in the use of sclerotherapy only for bleeding varices and not for
There is increasing evidence that combining a beta-blocker with a nitrate may be more effective in lowering portal pressure 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. 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. 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. 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, with 82% versus 72% of patients stopping bleeding and appears to be comparable in effectiveness to injection sclerotherapy 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.\textsuperscript{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.\textsuperscript{152} The management of bleeding gastric varices was previously restricted to surgery with shunting or gastric devascularization but the development of transcutaneous intrahepatic porto-systemic 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.\textsuperscript{153} Portal hypertensive gastropathy has been shown to worsen with injection sclerotherapy and ablation of oesophageal varices.\textsuperscript{154} Treatment is generally unsatisfactory and is usually with vasopressin and octreotide. Rectal varices\textsuperscript{155} are well recognized and rarely give major clinical problems. Injection therapy can be used to good effect. Portal hypertensive colopathy\textsuperscript{156} 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 porto-systemic shunt**

A major development in the management of portal hypertension has occurred with the advent of transcutaneous intrahepatic porto-systemic shunts (TIPS). This was a development of prior transjugular techniques developed for embolization of gastric and oesophageal varices.\textsuperscript{157,158} The first description of an intrahepatic shunt using a balloon catheter was in 1982\textsuperscript{159} but the initial results were poor as the dilated tract through the liver substance quickly closed resulting in recurrent varical bleeding.\textsuperscript{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.\textsuperscript{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\textsuperscript{162–164} involves transjugular 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.\textsuperscript{165} The Wallstent is easier to place and is potentially removable,\textsuperscript{166} although in practice this is rarely attempted. The technical advances and increasing expertise allow placement in approximately 90% of patients\textsuperscript{162} and the procedure lasts from 30 minutes to 2 hours in experienced hands.\textsuperscript{167} Complications of the procedure are infrequent and consist of bleeding into either biliary tree or abdominal cavity,\textsuperscript{168} renal failure due to the high-contrast load,\textsuperscript{167} migration of stents\textsuperscript{168} into pulmonary or systemic circulation which usually produce few symptoms. The only catastrophic complication is extrahaepatic 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 pressure\textsuperscript{169} and in the majority of patients the portal to hepatic vein pressure gradient was reduced to below 12 mmHg,\textsuperscript{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.\textsuperscript{170} Variceal rebleeding occurs in about 15% of patients at 1 year, due to stent occlusion by thrombosis or intimal hyperplasia\textsuperscript{164} 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.\textsuperscript{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.\textsuperscript{171} Age and shunt diameter are predictors of post-TIPS encephalo-
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. Long-term survival appears to relate to the severity of underlying liver disease.

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 described 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. 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. 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. 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.

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, 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. 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 scale 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 re-infection 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 and the 2 year survival is only 45%. If the patient has replicating HBV, as judged by PCR for HBV-DNA in the serum, the risk of re-infection 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.

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. Co-infection with the delta virus appears to be associated with less aggressive liver disease after transplantation. 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. 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. There is no doubt, however, that many patients with HCV infection do not produce antibodies to the virus post-transplantation. Of patients with pretransplant HCV antibodies, viraemia is present in 95% after transplantation and some patients went on to develop cirrhosis. This accords with our own experience, recurrent HCV appears to be common and, although frequently producing only mild liver disease, can run a severe course leading to
cirrhosis and retransplantation. 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. Similar figures have been reported from other centres. 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 chemotherapy, but the King's College series showed no major advantage for neo-adjuvant chemotherapy using doxorubicin and controlled trials are required. Patients transplanted for cholangiocarcinoma complicating primary sclerosing cholangitis whether diagnosed pre-operatively or at the time of surgery have a poor prognosis. 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. 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. 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. Treatment is usually by balloon dilatation or stenting but these procedures rarely produce long-term patency, and biliary surgery or retransplantation are often required.

### Immunosuppression for liver transplantation

Cyclosporin A has been a major factor in the improvement of results of liver transplantation during the past decade 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. Patients with early chronic or severe acute rejection could be rescued by changing therapy to FK506 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.

### 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. Other studies have shown a trend for a reduction in graft rejection with class I HLA matching. 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.

### 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 agent228,229 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number</th>
<th>12 month survival</th>
<th>24 month survival</th>
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</thead>
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<tr>
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<td>41%</td>
<td>6%</td>
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<td>ADM + LIP + TAE</td>
<td>25</td>
<td>56%</td>
<td>37%*</td>
</tr>
<tr>
<td>CIS + LIP + TAE</td>
<td>52</td>
<td>71%</td>
<td>46%*</td>
</tr>
</tbody>
</table>

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 enthanol 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 described\(^{243}\) 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 \textit{in vitro} and \textit{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 alphafeto-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 literature\(^{252}\) 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 death\(^{253}\) 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 B\(^{257}\) and C infection,\(^{258,259}\) cirrhosis\(^{260}\) 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 \textit{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 model\(^{265}\) found a p53 mutation (but not a codon 249 mutation) in 1/4 HCCs in rhesus and cynomologous monkeys. Other authors have suggested that HBV infection \textit{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.
Hepatitis A virus


Hepatitis B virus


LIVER DISEASE 179


Hepatitis C virus


Hepatitis D and E


Metabolic liver disease


126. Eriksson, S. & Larsson, C. Purification and partial charac-


**Portal hypertension**


145. Garcia-Pagan, J.C. & Feu, F. Sibai, B. & Rodes, J. Pro-
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147. Gimson, A.E.S., Ramage, J.K., Panos, M.Z. et al. Rando-


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180.


LIVER DISEASE


Hepatocellular carcinoma


Liver disease.

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