

personal communication).⁵ There is little justification for the use of immunoglobulin preparations in family contacts of patients, as the donated serum is almost invariably from First-World countries where most of the population has not been exposed to HEV.³ Hepatitis E is a self-limiting disease. Liver biopsies taken years after the illness show no signs of chronic disease.²⁶

Prevention

Hepatitis E is found worldwide but is particularly prevalent in developing countries where living conditions, water supply and solid waste disposal are poor.¹ Improved provision of clean water and adequate sanitation reduce the spread of HEV. The sequencing of the genome has provided valuable information which could lead to the successful production of a vaccine against this disease.

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Hepatitis viruses and hepatocellular carcinoma

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Of the hepatitis viruses that have been identified and their pathological consequences characterised, three — hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV) — have been implicated as risk factors for hepatocellular carcinoma (HCC) in humans. Sufficient evidence is now available to justify the conclusions that chronic infection with HBV and HCV, but not HDV, are causes of HCC. Hepatocellular carcinogenesis is a complex step-wise process that evolves over many years, and the precise way(s) in which these two viruses induce malignant transformation remain uncertain. The observation that HBV DNA is integrated into cellular DNA in the great majority of, and perhaps all, HBV-related HCCs, whereas replicative intermediates of HCV do not insert into host DNA in HCV-related HCC, makes it very likely that different pathogenic mechanisms operate in HBV- and HCV-induced HCC. Indeed, evidence is mounting that both direct and indirect mechanisms, and often the two together, are involved in the genesis of HBV-related HCC, but that HCV appears only to induce HCC indirectly by causing chronic necro-inflammatory hepatic disease which in turn is responsible for tumour formation. There is some evidence that the two viruses may interact in the development of HCC, but this remains to be proven. Animal models — other members of the hepadnavirus family (to which HBV belongs) that also cause HCC in their respective animal hosts, and transgenic mice into which sequences of HBV DNA have been inserted — are proving useful in elucidating putative mechanisms of HBV-related hepatocellular carcinogenesis, but no models for studying HCV-induced HCC are yet available. Whatever the pathogenesis of HBV-induced and HCV-induced HCC, the viruses do not act alone but in conjunction with other environmental carcinogens and a number of host factors.

Evidence for a causal role for hepatitis viruses in HCC

HBV

The evidence linking chronic HBV infection to the development of HCC is now well established and needs only brief review.

1. With few exceptions, the geographical distributions of the HBV carrier state and HCC, at both a global and a more local level, parallel each other closely.¹

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2. Individuals who become carriers of the virus at an early age have a lifetime relative risk of developing HCC greater than 100, and nearly 50% die from HCC, cirrhosis or both.² In regions with the highest incidences of HCC — sub-Saharan Africa and parts of the Far East — infection with HBV occurs in the first few months of life as a result of either perinatal or horizontal transmission (about 90% of children born to mothers with replicative HBV infection become chronic carriers of the virus).² This group has the greatest chance of developing HCC. The risk for those infected as adults has not been formally ascertained, but it is certainly substantially less than that recorded in early-onset carriers.² This is illustrated, for example, by a recent follow-up study of American military personnel who developed acute B virus hepatitis during World War II; surprisingly few subjects progressed to chronic hepatic disease and even fewer to HCC.³ Most of the approximately 300 million carriers of HBV worldwide acquire the infection early in life.

3. The presence of the HBV carrier state almost always precedes the onset of HCC by many years, an interval commensurate with a cause-and-effect relationship between the virus and the tumour.¹ The probability of HCC occurring increases progressively with the duration of chronic HBV infection.

4. Although the proportion of patients with HCC who have markers of current HBV infection differs appreciably in different geographical regions, the patients, whether in regions with high, intermediate, or low incidences of the tumour, consistently have a higher prevalence of these markers than do appropriate controls.⁴ Based on these data, chronic HBV infection has been estimated to cause as much as 80% of global HCC.

5. Almost all HCCs occurring in regions where the HBV carrier state is endemic contain integrated sequences of viral DNA.⁵

6. Indirect evidence for a causal link between HBV and HCC is provided by the observation that other hepadnaviruses also induce HCC in their respective hosts (in woodchucks, with a frequency of close to 100% within 3 years of infection, and in ground squirrels, around 30% within 5 - 6 years).^{6,7} Integrated sequences of hepadnaviral DNA are present in cellular DNA in the tumours of both these models.^{6,7}

HCV

Evidence of an aetiological role for HCV in HCC is more recent but nevertheless persuasive. The first hint of an association between chronic HCV infection and the occurrence of the tumour was provided by isolated reports of patients⁸⁻¹² (and a chimpanzee¹³) with transfusion-acquired non-A, non-B hepatitis who, over a period of many years developed, in turn, chronic hepatitis, cirrhosis, and finally HCC (serum from some of these patients, but not the chimpanzee, was later used to verify infection with HCV). With the recent availability of serological tests for HCV, it has become possible to investigate the association between HCV and HCC more thoroughly. First-generation enzyme-linked assays, which detected antibody to a single HCV epitope (C-100), were of limited specificity and sensitivity, and were soon superseded by assays which recognise additional antibodies against both structural and non-

structural HCV epitopes and reflect more reliably the presence of anti-HCV in serum. Furthermore, recombinant immunoblot assays (RIBAs) are now used as confirmatory tests. More recently, HCV RNA in serum and tissues was amplified by means of the polymerase chain reaction (after the RNA was converted to DNA with reverse transcriptase); properly done, this method provides the most accurate — although not infallible — means of evaluating the closeness of the correlation between persistent HCV infection and the formation of HCC.

As is true of HBV, the proportion of patients with HCC who have circulating anti-HCV shows a pronounced geographical variation. In Japan,¹⁴⁻¹⁶ which has a high incidence of HCC, and in Italy^{17,18} and Spain,^{19,20} with intermediate incidences, between 47% and 83% of the patients with HCC have circulating anti-HCV. The relative risk for HCC in Japanese with persistent HCV infection is 52,3 (95% confidence interval 23,9 - 114,3)¹⁵ and in Italians 69,1 (15 - 308).¹⁸ These risks are 3 - 4 times higher than those for chronic HBV infection in the same populations, and markers of current HBV infection are present in only 20 - 25% of these patients. During recent decades the incidence of HCC in Japan has increased substantially, and this has been attributed to an increasing frequency of chronic HCV infection in this population.²¹

In those geographical regions where HBV infection is endemic and this virus is the major risk factor for HCC, anti-HCV is present in the serum of only a minority of patients with the tumour — in Far Eastern countries between 6% and 39%,²²⁻²⁶ and in sub-Saharan Africa 20%.²⁷ Relative risks in these populations were calculated to be 7 (1,6 - 30,8) in Taiwan²² and 6,1 in Senegal (0,5 - 69).²⁸ Comparatively low prevalences of anti-HCV (13 - 35%)²⁹⁻³⁵ have also been recorded in the remaining regions for which information is available. Relative risks in these populations are 10,4 (4,2 - 26) in Greece²⁹ and 10,5 (3,5 - 31,3) in North America.³⁵ These prevalences and risks are similar to those for HBV in these regions. The global pattern of HBV- and HCV-related HCCs is shown in Fig. 1.

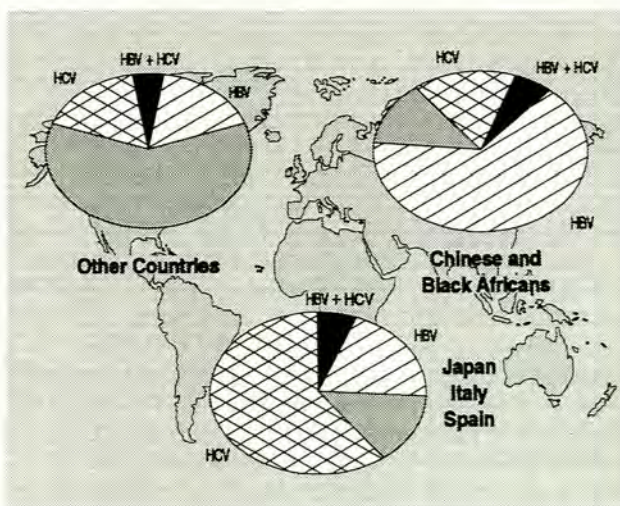


Fig. 1. The pattern of HBV-induced and HCV-induced hepatocellular carcinoma in different geographical regions and populations. The dark grey stippled portion of the pie diagrams represents aetiological factors other than HBV and HCV.

Two longitudinal studies of the risk of HCC developing in patients with HCV-related chronic benign hepatic disease have been published. Sixty-two Japanese patients with chronic hepatitis, whose serum was positive for anti-HCV and who were not alcoholics, were observed for at least 5 years.³⁶ Sixteen patients (25,8%) developed HCC during this time. Of these, 13 progressed to cirrhosis and 3 remained with chronic hepatitis. No control group was included. Patients with chronic benign hepatic disease with and without circulating anti-HCV were monitored in Sweden for up to 11 years.³⁷ The proportion of deaths from HCC was significantly higher in the anti-HCV-positive (18%) than in the anti-HCV-negative patients (4%). In a follow-up of 568 subjects who had had acute post-transfusion non-A, non-B hepatitis (no anti-HCV testing was done) and 968 matched controls a mean of 18 years after being transfused, only 1 case of HCC was uncovered in the hepatitis group and 2 among the controls (relative risk 1,0).³⁸ This finding may not be valid because of the relatively short period of follow-up after acute hepatitis.

HDV

There is at present no convincing evidence of a causal role for HDV in HCC. Only 3 case-control studies of the seroprevalence of antibody to the Delta antigen (anti-HDV) in patients with HCC have been documented (although 14 case series have been published), and none of the 3 showed the presence of anti-HDV to be a risk factor for tumour over and above that attributed to concurrent chronic HBV infection.³⁹⁻⁴¹ One possible explanation is that patients with combined HBV/HDV infection have such severe chronic necro-inflammatory hepatic disease that they die before HCC has had time to develop.⁴²

Age and gender differences in HBV- and HCV-virus-induced HCC

In regions where HBV infection is endemic, HCC occurs at a younger age in patients with HBV-related tumours than in those with HCV-related tumours.^{24,27,43} The mean age of Taiwanese patients with anti-HCV-positive HCC is $65,1 \pm 6$ years compared with $55,5 \pm 11,9$ years in those with HBsAg-positive HCC;⁴³ in southern African blacks the corresponding figures are 52,3 years (range 37-78 years) and 40,3 years²⁷ (17 - 80 years), and in Hong Kong Chinese the mean difference is 7 years.²⁴ Significant age differences have also been recorded in some countries with a lower prevalence of the HBV carrier state: anti-HCV-positive patients in Japan have a mean age of $62,7 \pm 8,6$ years compared with $55,9 \pm 9,6$ years in HBsAg-positive patients¹⁴, and in the USA the corresponding figures are 59 years (range 24 - 76 years) and 44 years (32 - 52 years).⁴⁴ One possible explanation for these age differences is that, in these populations at least, HCV infection is acquired later in life than is chronic HBV infection. In other countries no age difference was apparent.¹⁷⁻¹⁹ The co-existence of HBV and HCV infections in patients with HCC does not, at least in Taiwanese patients, accelerate the rate of tumour development in comparison with HBV infection alone.⁴³

In most reports no difference in anti-HCV positivity rates was evident between men and women with HCC.^{17,19,44} However, in one Japanese¹⁵ and one Italian⁴⁵ study, circulating anti-HCV was present significantly more often in males, and in an analysis from the west coast of the USA the relative risk of HCC developing in anti-HCV-positive men (19,6) was appreciably greater than in anti-HCV-positive women (5,1).³⁵ Anti-HCV-positive HCC may be relatively more common than HBsAg-positive HCC in women in Hong Kong (female/male ratio — 1:7 compared with 1:11)²⁴ and in the USA (1:6,7 compared with 0%:100%).⁴⁴ This appeared also to be true in southern African blacks when they were tested with a first-generation assay⁴⁶, but was not confirmed in a later study with a second-generation assay and measurement of HCV RNA.²⁷ No difference was apparent in Taiwanese patients.⁴³

Putative mechanisms of hepatitis virus-induced hepatocellular carcinogenesis

HBV

Evidence continues to accumulate that both direct and indirect mechanisms are involved in HBV-induced hepatocellular carcinogenesis.

Direct carcinogenicity

Although HBV-related HCC often co-exists with cirrhosis⁴⁷ (and sometimes with chronic hepatitis), in a proportion of patients the tumour arises in an otherwise normal liver, and it is in these patients that a specific carcinogenic effect is most likely. Moreover, in populations with a high incidence of HBV-related HCC, HBsAg is present in serum and hepatic tissue as often in those HCC patients without cirrhosis as in those with co-existing cirrhosis.^{48,49} The fact that woodchucks chronically infected with woodchuck hepatitis virus (WHV) show a gradual transition from preneoplastic nodules to fully developed HCC in the absence of cirrhosis or chronic hepatitis provides further, albeit indirect, support for a direct mechanism.⁵⁰

The HBV genome does not contain a known oncogene, and the long interval between infection and the appearance of HCC confirms that HBV does not code for an acutely transforming protein. However, the detection of HBV DNA integrants in host DNA in about 85% of reported HBV-related HCCs (95% in regions where HBV and HCC are common)⁵ is consistent with the mechanism of tumorigenesis described with non-acutely transforming viruses, namely insertional mutagenesis. Integration could even be invariable in these tumours: cellular DNA flanking HBV DNA inserts often becomes grossly rearranged as a result of deletions, duplications, amplifications and translocations, and in those tumours without HBV DNA the integrant or integrants could subsequently have been lost as a result of deletion. Such an occurrence would imply that integrated HBV DNA, even if essential for the initiation of hepatocellular carcinogenesis, is not needed for the maintenance of the transformed phenotype ('hit-and-run' hypothesis). Other integrants could be too short to be detected by current methods.

Insertion of HBV DNA into cellular DNA appears to occur at random sites, although some chromosomes are affected more often than others.⁵¹ This observation argues against integration, either in or near the regulatory elements of a proto-oncogene (or other gene concerned with cell growth, cycling or differentiation) or within a tumour suppressor gene, being a numerically important way of inducing malignant transformation of hepatocytes. Indeed, there are very few reports in the literature of HBV DNA integration near a growth regulatory gene^{52,53} and none of disruption of a tumour suppressor gene in human HCCs. Because most HCCs contain multiple integrants and these studies have been undertaken only when a single integrant was present, and because other genes adjacent to sites of viral insertion may yet prove to be growth regulatory or tumour suppressor genes, the frequency of direct insertional mutagenesis may be underestimated. Indirect support for the potential importance of this mechanism is the finding that in about 50% of HCCs in woodchucks infected with WHV, integration takes place within regulatory sequences of c-myc or N-myc 2; this leads to activated expression of these genes.^{54,55} Furthermore, transgenic mice into which the woodchuck c-myc gene in tandem with upstream WHV DNA has been directly inserted, develop HCC.⁵⁶ However, when ground squirrels infected with ground squirrel hepatitis virus were studied by the same laboratory, none of the integrants was found to be in the region of c-myc or N-myc 2 (W. S. Robinson and P. Tiollais — personal communication), so that the relevance of the finding to human HCC is difficult to assess. Moreover, in a transgenic mouse model expression analysis of all proto-oncogenes and tumour suppressor genes thus far known to be associated with hepatocellular carcinogenesis failed to reveal any qualitative or quantitative alteration.⁵⁷ Activated c-myc, N-ras and c-fos expression have been reported in human HCCs, but expression of these oncogenes is increased during hepatic regeneration and these changes may be a consequence rather than the cause of cellular proliferation in the tumour.⁵⁸ Nevertheless, mutations of codon 6 of N-ras and codon 12 of H-ras have occasionally been reported.^{51,59}

Unique fusion proteins encoded by adjacent virus and host DNA sequences and having transforming properties play a crucial role in retrovirus-induced cancers.⁶⁰ Such fusion proteins have, however, not been detected in human HCCs. Although there is no proof that insertion of HBV DNA has directly resulted in disruption of tumour suppressor genes, loss of heterozygosity on chromosomes 4q, 11p and 17p (including some in the region of the p53 tumour suppressor gene) has been described in relation to HBV integration.⁶¹ Loss or disruption of chromosomal DNA secondary to viral integration may also have effects on the expression of proto-oncogenes or tumour suppressor genes remote from the site of integration.^{62,63}

Integrated HBV DNA may induce malignant transformation in another way. The product of the HBV X gene can activate *in trans* a wide variety of viral and cellular promoters, thereby perturbing the expression of cellular genes and the differentiated functions of the infected cell in such a way as to lead ultimately to malignant transformation of the cell.^{64,65} Evidence in support of this putative mechanism has recently been published. The entire X gene, under its own regulatory elements, was placed into the germline of mice. The

transgenic mice harbouring the gene developed, in turn, multifocal areas of altered hepatocytes, benign hepatic adenomas and finally malignant carcinomas, all of this in the absence of chronic necro-inflammatory hepatic disease.⁶⁶ This sequence of events is the same as that described in experimental chemical carcinogenesis, and suggests that abnormal expression of cellular genes may initiate aberrant growth. The X gene, *in toto* or in a 3' truncated form, is often included in HBV DNA integrants, and could express X polypeptides or X-cellular fusion proteins.^{67,68} Three truncated preS/S genes have also been shown to have *trans*-activating properties.⁶⁹ (This region is most often included in HBV DNA integrants.) Activation or inactivation of growth regulatory genes in hepatocellular carcinogenesis, whether *in cis* or *in trans*, remains an attractive hypothesis because either could confer upon initiated hepatocytes a growth advantage which, coupled with an opportunity for selection, would generate cells that are at risk for further genetic events in the multistep process of tumour formation.

Insertion of HBV DNA into cellular DNA has not, however, been proved to be an essential first step in virally-induced carcinogenesis. If extra-chromosomal DNA can be directly carcinogenic, the way in which this would be accomplished is not known. On the other hand, integration is not synonymous with malignant transformation because HBV DNA integrants have been detected in cirrhotic nodules.⁷⁰ Neither is it known if HBV can cause HCC in the absence of other environmental carcinogens. The most convincing evidence that it can is indirect: woodchucks infected with WHV as neonates develop HCC even when reared in an environment free of known carcinogens.⁴⁹ The only evidence in humans is the observation that HBsAg-positive mentally deficient children growing up in an institution in California, USA, in the absence of aflatoxin and other known chemical carcinogens, have a relative risk for HCC of more than 200.⁷¹ Of significance also was the observation that these tumours arose in the absence of cirrhosis.

Indirect carcinogenicity

The majority of HCCs throughout the world co-exist with cirrhosis.⁴⁷ Moreover, all forms of cirrhosis, whatever their cause, may be complicated by HCC.⁴⁷ Accordingly, cirrhosis *per se* is regarded as a major risk factor for HCC. In studies carried out in the UK⁷² and Austria,⁷³ both of which have a low incidence of HBV-related HCC, cirrhosis (as well as male sex and the ages of the patients), but not the presence of HBsAg, proved to be a risk factor for HCC. Indirect evidence for an important role for chronic necro-inflammatory hepatic disease and the resulting hepatocyte regeneration in the pathogenesis of HCC is provided by the transgenic mouse model described by Chisari *et al.*⁷⁴ In this experiment the region of the HBV genome encoding for the surface and presurface proteins was inserted directly into the germline of mice. These mice overproduce large-envelope (preS1) protein, which accumulates in the endoplasmic reticulum of hepatocytes producing severe and prolonged hepatocyte injury that initiates a response characterised by inflammation, regenerative hyperplasia and transcriptional dysregulation, with ultimate progression to neoplasia. This finding supports the notion that continuous or recurrent regeneration of hepatocytes caused by persistent HBV infection provides an opportunity for the selection of cells

that possess some growth advantage, a crucial step in the promotion of tumour development.

Carcinogenesis is a complex step-wise process, and hepatocellular carcinogenesis is no exception. Because HCC is closely associated with chronic HBV infection rather than HBV infection *per se*, the molecular genetic events essential to HCC formation must be related to continuous or recurring cycles of viral infection. Indeed, it is very likely that indirect and direct effects of HBV act together in the induction of most HBV-related HCCs. The increased hepatocyte turnover rate, resulting from continuous or recurring cycles of viral infection with consequent cell necrosis and regeneration, increases the likelihood both of viral integration and of the resultant changes in DNA being 'fixed' in the daughter cells, as well as providing an opportunity for the selective growth advantage of the initiated cells to be exercised. Another possible way in which indirect and direct carcinogenic effects may interact concerns the enzyme topo-isomerase I. This cellular enzyme alters the superhelical state of DNA by nicking-closing reactions on single- and double-stranded DNA.^{75,76} With double-stranded DNA there is no resultant conformational change, but when the DNA is single-stranded topo-isomerase I cleavage causes linearisation of the DNA. Topo-isomerase I has been shown to be capable of generating WHV DNA integration into cellular DNA *in vitro*.⁷⁷ Intracellular concentrations of the enzyme are increased during cell replication.⁷⁸ Thus, the accelerated cell turnover rate accompanying virally-induced chronic necro-inflammatory hepatic disease may, by enhancing topo-isomerase I activity, result in cleavage of viral DNA at specific sites and promote its insertion into cellular DNA. There is also epidemiological evidence that favours a combination of carcinogenic effects in that HCC is far more likely to occur in cirrhotic patients who are HBsAg-positive than in those who are HBsAg-negative,⁷⁹ and in HBV carriers with cirrhosis or chronic hepatitis than in those without chronic hepatic disease.⁸⁰ Furthermore, the occurrence of HBV-related HCC in children is linked to the rapid development of cirrhosis.⁸¹

HCV

The pathogenesis of HCV-induced HCC is unknown. Although HCV, a positive-stranded RNA virus, has been shown to replicate in HCC tissue,⁸² there is as yet no evidence that replicative intermediates of the viral genome become integrated into host DNA. If this observation is confirmed, insertional mutagenesis can be excluded as a pathogenic mechanism. Nor is there, at present, any evidence that HCV genes have *trans*-activating properties. Based on current knowledge, it thus seems unlikely that HCV is directly carcinogenic. This conclusion is supported by the fact that no well-documented case of HCV-induced HCC arising in a normal liver has yet been reported. In published series the non-tumorous hepatic tissue in the great majority of these patients has been cirrhotic and the remainder have had chronic hepatitis. A few reports — on Italian^{18,45} and Taiwanese⁸³ patients — have stated that a small proportion of the anti-HCV-positive patients with HCC have not had cirrhosis, but the authors failed to mention

whether the non-neoplastic tissue showed chronic hepatitis or was normal. However, in one report on Italian patients, the authors write that 'a few' patients with HCC and a normal liver had circulating anti-HCV detected by a first-generation assay.⁸⁴ Infection with HCV is often complicated by progression to chronic hepatitis and cirrhosis.⁸⁵ Taken together, these observations strongly support the notion that HCV acts indirectly in the pathogenesis of HCC, i.e. that chronic HCV infection causes chronic necro-inflammatory hepatic disease and it is this rather than the presence of the virus *per se* that is responsible for subsequent tumour formation. Ongoing or recurrent virally-induced hepatocyte necrosis and regeneration increase cell turnover rates, thereby rendering the DNA more susceptible to spontaneous or mutagen-induced changes.

Because the great majority of HCV-related HCCs arise in cirrhotic livers,⁴⁷ the question arises whether chronic HCV infection enhances the risk of the development of HCC in cirrhotic livers, whatever their cause. Simonetti *et al.*¹⁸ showed anti-HCV to be present in 74% of Italian patients with HCC and cirrhosis but in 62% of those with cirrhosis alone (odds ratio 1.8; range 1.1 - 2.8). They concluded that HCV increases the risk of HCC in patients with cirrhosis, irrespective of the aetiology. Caporaso *et al.*⁸⁶ found circulating HCV (as well as age and male sex) to be a risk factor for HCC in Italian patients with cirrhosis. Anti-HCV was present significantly more often in Spanish patients with alcoholic cirrhosis and HCC (54%) than in those with alcoholic cirrhosis alone (24%),⁸⁷ but there was no difference in patients with cryptogenic cirrhosis.¹⁹ Similar findings in respect of alcoholic cirrhosis were reported in French patients,⁸⁸ although in a multidimensional analysis of risk factors for HCC in French patients with alcoholic cirrhosis, anti-HCV seropositivity proved to be only weakly associated with tumour formation.⁸⁹ These findings imply that HCV may play a role in inducing HCC formation in cirrhotic livers that have resulted from alcohol abuse, although the evidence in respect of other aetiological forms of cirrhosis is equivocal.

Possible interaction of HBV and HCV in the genesis of HCC

An interaction, if any, between HBV and HCV in hepatocellular carcinogenesis remains uncertain. Most early studies showed circulating anti-HCV to be present appreciably more often in HBsAg-negative than in HBsAg-positive patients with HCC.^{14,15,21,32,43,45,83,84} Similarly, in a recent analysis of black Africans the prevalence of current HBV infection was significantly lower in patients positive for HCV viraemia than in those who were negative.²⁷ These observations suggested that HCV was particularly likely to be a causative factor in patients in whom HBV could not be implicated. However, other case series and case control studies have not shown a difference.^{17,19,87} In only a few reports have relative risks for HCC in individuals positive for anti-HCV alone, HbsAg alone, and the two together been calculated. In a study of anti-HCV-positive Greek patients, which permitted the examination of mutual confounding and interactive effects between HCV and HBV, Kaklamani *et al.*⁹⁰

calculated a relative risk for HCC of 20 in HBsAg-positive subjects compared with only 4.8 in those without this marker. This confirmed an earlier observation from the USA.³⁵ The relative risk for HCC in Taiwanese subjects positive for both HBV and HCV was 40, compared with 14 in carriers of HBV alone and 27.1 in carriers of HCV alone.³¹ Using a slightly different approach, Yuki *et al.*¹⁶ assayed for anti-HCV in Japanese carriers of HBV, some of whom suffered from HCC. The antibody was present substantially more often in the carriers with tumour (32%) than in those without (5%). Assuming that HCV infection is usually acquired earlier in life than is HBV infection, these studies suggest that HCV superinfection in HBV carriers increases the risk of HCC development. However, in a report of Italian patients multivariate conditional logistic regression showed that HCV and HBsAg act as completely independent risk factors.¹⁸ Neither could a statistically significant synergism between markers of HCV and HBV be found in French patients with HCC.⁶⁸ These discrepant findings may result from the small numbers of patients with both HCV and HBV serological markers included in the studies. Larger analyses in regions with different rates of chronic infection with HBV and HCV will be required in order to determine if there is an interaction between the two viruses in the genesis of HCC.

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The laboratory diagnosis of acute viral hepatitis

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The definitive diagnosis of viral hepatitis depends on the demonstration of the virus or of serological markers of recent infection. The serological tests to establish the aetiology of viral hepatitis vary from laboratory to laboratory. Those commonly performed are discussed here. An algorithm (Fig. 1) is provided as a guide to the investigation of patients with suspected hepatitis. It is stressed that the choice of initial tests should be based on the clinical findings in each individual patient.

Hepatitis A

Hepatitis A virus (HAV) or viral antigen can be detected in stool and other body fluids by various techniques including electron microscopy and molecular hybridisation to radiolabelled cDNA probes or single-stranded RNA (viral RNA) probes. However, these techniques are time-consuming and expensive. By the time the patient presents with symptoms, the infection is established and the diagnosis is thus based on the detection of specific antibodies to HAV by radio-immunoassay (RIA)¹ or enzyme-linked immunoassay (ELISA).²

IgM anti-HAV antibodies are detectable in the serum at the onset of symptoms, the titres rise rapidly and may persist for 48 - 60 days after the onset of symptoms. False positives are rare. The presence of HAV IgG antibody alone indicates previous exposure and thus immunity, and excludes current infection.

Hepatitis B

Commercially available tests for the diagnosis of hepatitis B virus (HBV) infection include tests for the detection of HBsAg, HBeAg, HBV DNA (by hybridisation techniques), anti-HBs, anti-HBe, IgM anti-HBc and IgG anti-HBc. For research purposes, tests are available to detect HBV DNA by polymerase chain reaction (PCR), HBV DNA polymerase activity, pre-S₁ and pre-S₂ antigens and their antibodies.

In acute hepatitis B, HBsAg is detectable during the prodromal phase prior to the elevation of transaminases. Although the presence of HBsAg in the serum implies active infection, the absolute level has no clinical significance and does not correlate with the degree of infectivity.³ However, in an individual patient, a decreasing HBsAg titre is indicative of a resolving infection. In a minor infection, HBsAg is cleared rapidly and the only evidence of infection may be

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