

The elusive hepatitis C virus

A cause of parenteral non-A, non-B hepatitis

Specific laboratory tests are generally available now for three hepatitis viruses: hepatitis A (infectious or epidemic hepatitis), hepatitis B (previously referred to as serum hepatitis), and hepatitis D (the delta virus). A fourth virus, which causes enterically transmitted non-A, non-B hepatitis (or epidemic non-A hepatitis) has been seen by electron microscopy and has morphological and biophysical characteristics that suggest that it is a member of the calicivirus group. Serological tests are under development, and molecular cloning of this virus is in progress. Other viruses that cause an acute hepatitis include the Epstein-Barr virus, cytomegalovirus, yellow fever virus, and the more exotic haemorrhagic fever viruses such as Lassa fever virus, the Marburg agent, Ebola virus, and Rift Valley fever virus, but the diseases caused by these viruses are not generally included in the term viral hepatitis.

As specific diagnosis of the different types of viral hepatitis became possible some 10 years ago it became apparent that there was one form, previously unrecognised and unrelated to hepatitis A, B, or D, that was transmitted principally by the parenteral route. This became known as non-A, non-B hepatitis. Results obtained from several carefully conducted surveys of posttransfusion hepatitis in the United States and elsewhere provided strong epidemiological evidence of "guilt by association." This form of non-A, non-B hepatitis has been found in every country in which it has been sought; and it shares several features with hepatitis B. In countries where all blood donations are screened for hepatitis B surface antigen by very sensitive techniques non-A, non-B hepatitis may account for as many as 90% of all cases of posttransfusion hepatitis. Outbreaks of non-A, non-B hepatitis have also been reported after treatment with blood clotting factors VIII and IX. Non-A, non-B hepatitis has occurred in haemodialysis and other specialist units, among drug addicts, and after accidental inoculation with contaminated needles and other sharp objects. Mother to infant transmission has been reported.¹ In Britain and in several other countries some cases are not associated with transfusion, and such sporadic cases of non-A, non-B hepatitis account for 10-25% of all adult patients with clinical viral hepatitis. The route of infection or the source of infection cannot be identified in many of these patients, but sexual transmission is a strong candidate.²

Although in general the illness is mild and often subclinical or anicteric, severe hepatitis with jaundice does occur, and non-A, non-B hepatitis accounts for a substantial fraction of all fulminant hepatitis. In many patients the infection may be

followed by prolonged viraemia and a persistent carrier state. Studies of the histopathological sequels of acute non-A, non-B hepatitis infection showed that chronic liver damage, which may be severe, may occur in as many as 40-50% of patients.

The virus(es) causing the parenterally transmitted form of non-A, non-B hepatitis have not been fully characterised, and their mode of replication and antigenic composition were not clear until recently despite intensive efforts in many laboratories throughout the world. There is no homology between the viruses causing hepatitis A and B, the delta hepatitis virus, and the enterically transmitted and the parenteral non-A, non-B virus.

A claim to have identified the last of these viruses came in the form of a news release by the Chiron Corporation, based in California, followed by a press conference in Washington in May 1988. This described the identification, cloning, and expression of proteins from the virus (*Wall Street Journal*, 11 May 1988). But, to the intense disappointment of the medical and scientific community, no scientific publication appeared until nearly a year later.

Choo *et al* derived a random primed complementary DNA from infectious plasma known to contain the non-A, non-B agent using a construct in the bacteriophage λ gt 11.³ This phage vector permits efficient expression of polypeptides encoded by complementary DNA and was designed originally by Young and Davis to help isolate complementary DNA clones by well characterised antibodies that bind to clones synthesising the polypeptides of interest.⁴ The infectious plasma was ultracentrifuged to make a pellet from a putative small virus, and nucleic acid was recovered from this. Since the nature of the genome of the virus was not known, the nucleic acid was denatured before synthesising complementary DNA from both RNA and DNA with random primers of reverse transcriptase.

About a million clones were screened. One was found to produce a protein that reacted with the serum of a patient with chronic non-A, non-B hepatitis, which was presumed to contain antibodies to the virus. The 155 base pair insert in this clone was then cut and used as a hybridisation probe to the original library of complementary DNA, and a larger overlapping clone with 353 base pairs was extracted. This double stranded cloned DNA did not hybridise to host cell DNA or to DNA derived from plasma or liver infected with non-A, non-B hepatitis. One of the strands hybridised specifically to RNA from infected liver, however, and was shown to be homologous to a single stranded RNA in the original

pellet obtained by ultracentrifugation of infectious plasma. These and other data indicated that the three overlapping clones contained at least part of the bloodborne non-A, non-B hepatitis virus. The virus, now termed hepatitis C virus, contains 10 000 nucleotides of a single stranded RNA molecule in one common open reading frame.

The continuous open reading frame was reconstructed and then expressed in yeast as a fusion polypeptide with human superoxide dismutase, which facilitates the efficient expression of foreign proteins in yeast and bacteria. A polypeptide containing 363 viral amino acids in large amount (about 4% of total protein) was expressed in the recombinant yeast. After solubilisation and purification the antigen was used to coat the wells of microtitre plates so that hepatitis C virus (HCV) antibodies could be captured and assayed by radioimmunoassay⁵ or enzyme immunoassay. The specificity of the test system for detecting anti-HCV antibodies was soon established by examining well documented panels of sera from both patients with posttransfusion non-A, non-B hepatitis and implicated blood donors. Antibodies were also found in some sera from late convalescent patients with sporadic, community acquired non-A, non-B hepatitis in several countries.^{5,9}

A similar molecular cloning technique was used independently and successfully for isolating the bloodborne non-A, non-B hepatitis virus by Dr Terukatsu Arima and colleagues at the Okayama University Medical School (paper to the American Association for the Study of Liver and to a WHO meeting in London, 1988),¹⁰ and reportedly by another group also working in Japan. Several clones isolated by Arima *et al* expressed proteins that have been shown in an independent laboratory to react specifically with well documented panels of non-A, non-B hepatitis (T Arima, personal communication). Sadly and surprisingly, no direct comparisons of the cloned products obtained by the different laboratories have yet been reported—reflecting, presumably, an unwelcome feature of current economic doctrines, which seem to require research teams to secure patent protection before disclosure. This is often an unduly long process.

Nevertheless, the preliminary data on the nature of the hepatitis C virus are interesting. Previous studies carried out principally by Bradley *et al* and He *et al* showed that the candidate non-A, non-B hepatitis virus is less than 80 nm in diameter and sensitive to organic solvents, indicating a lipid containing viral envelope, and might be a togavirus.^{11,12} The work of Choo and his colleagues shows that the molecular organisation of HCV is indeed consistent with a virus related to the family of togaviruses or the flaviviruses, both of which are arboviruses—a group of viruses usually transmitted by insect bites.³ Though this is perhaps an unexpected finding for a common hepatitis virus, another arbovirus, yellow fever virus, is a well recognised hepatotropic virus. In another study Fagan *et al* detected virus-like particles (60-70 nm) with an envelope surface projection budding into cell vacuoles and rod shaped inclusions in nuclei of hepatocytes from several patients in Britain given liver transplants for sporadic non-A, non-B fulminant hepatitis.^{13,14} Identical particles were seen in two successive liver grafts (days 2 and 10) at re-grafting for recurrent fulminant hepatic failure. The ultrastructural features of these particles resembled those of the RNA containing arbovirus, Rift Valley fever virus, but the results of serological studies of many representative arboviruses and transmission in mice were negative. These findings provide further evidence that virus(es) resembling the arboviruses but not transmitted by an insect vector are concerned in the aetiology of non-A, non-B hepatitis, including the sporadic form of fulminant viral hepatitis in Britain.

The most recent published seroprevalence studies of anti-HCV antibodies using the assays developed by the Chiron

laboratories confirm the apparent specificity and (relative) sensitivity of the assays.^{5,9} Anti-HCV antibodies were found in up to 85% of patients with posttransfusion non-A, non-B hepatitis and in implicated donors, and there was a strong correlation between raised alanine aminotransferase activities and the presence of anti-HCV antibodies. Similarly, a high overall prevalence of anti-HCV antibodies has been reported in 60-80% or more of patients with haemophilia receiving maintenance treatment with replacement clotting factors, 60-70% of patients with chronic active hepatitis or cirrhosis with a history of blood transfusion, and 50-70% of intravenous drug abusers. In Spain anti-HCV antibodies were found in two out of 26 homosexual men infected with HIV and in one out of eight women contacts of drug abusers and, more surprisingly, in 15 out of 34 patients with autoimmune chronic active hepatitis and in 10 out of 26 patients with primary biliary cirrhosis, alcoholic cirrhosis, or cryptogenic cirrhosis, none of whom had a history of blood transfusion.⁶ Among 241 healthy pregnant women in Spain, 98 of whom were carriers of hepatitis B surface antigen, three had anti-HCV antibodies, but antibody was not detected in any of 49 randomly selected blood donors. Preliminary observations from Japan and Italy indicate a high prevalence of anti-HCV antibodies in patients with primary hepatocellular carcinoma, particularly in patients without detectable markers of hepatitis B virus.

The ability to detect anti-HCV antibodies, generally only several months after acute infection, is an important advance that is expected to provide not only a clinical diagnostic test but also a screening procedure for blood donations. Such a test would substantially improve the safety of the transfusion of blood and blood products. Preliminary serological surveys of healthy blood donors indicate average rates of anti-HCV antibodies in 0.5-1.0% in the National Blood Transfusion Service of Britain,¹⁵ with a similar rate in several other industrial countries that use a voluntary blood donation system.

Nevertheless, important problems remain. Many of these serological findings should be interpreted with some caution in the absence of a confirmatory test, sufficient independent testing, and more extensive testing, which is limited by the apparent lack of availability of reagents and test kits and the high cost of the reagents. The urgent and important problem is the lack of confirmatory assays for repeatedly reactive or borderline reactions by the commercial enzyme immunoassay in blood donations—although it has been argued, but unconvincingly, that the very nature of the reagents provides some assurance of specificity. In addition to this basic requirement, specific and sensitive assays are urgently needed for the different subclasses and early anti-HCV antibodies, for neutralising antibodies, and for detecting antigen and other viral products. We also need wider application of the tests to determine whether there is more than one type of bloodborne non-A, non-B hepatitis—for which there is much epidemiological, clinical, and experimental evidence.^{1,16,17}

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Peripheral vascular disease

Physical treatments may help

Intermittent claudication is usually caused by an arteriosclerotic stenosis that limits blood flow to the legs so that the increased demand during muscular work cannot be met. In the more advanced stages of occlusive peripheral vascular disease perfusion may no longer be adequate even at rest, and rest pain and eventually gangrene may result. Ideally the affected arteries should be reopened by surgery or fibrinolytic treatment. These methods are not, however, indicated in patients with the less severe forms of peripheral vascular disease; they are not possible in some patients and are unsuccessful in others.¹ In such cases the alternative treatments include drugs and haemodilution, both of which may alleviate symptoms.² A third possibility is physical treatment, which has the advantage of having fewer side effects.

Exercise is undoubtedly the most effective conservative treatment for patients with intermittent claudication. Controlled trials have shown that it doubles or even triples the distance that can be walked before pain occurs.^{3,4} Supervised long term treatment has a response rate of around 80%.⁵ Specific forms of exercise are sometimes helpful. They offer the advantage of "individualising" the treatment: depending on the site of the stenosis, the patient may (selectively) train those muscles that suffer the worst hypoxia.⁶

Most vascular surgeons advocate exercise only for patients with intermittent claudication, yet at least two studies have shown benefit for patients with rest pain.^{7,8} Various mechanisms seem to play a part in improving symptoms: haemodynamic changes help to redistribute the blood flow, metabolic alterations within the muscle cell optimise oxygen utilisation, and structural changes in the musculature affect both microvessels and muscle fibres. Other factors may be changes in walking technique, psychological responses, and improved rheological features in the blood.⁹

Various electrical treatments have been tried to extend the walking distance in patients with claudication.¹⁰ One trial of transcutaneous electrical nerve stimulation reported a 125% prolongation of the walking distance with stimulation compared with only 41% in the control group.¹¹ The mechanism of this effect could be either an analgesic effect or changes in blood flow. Transcutaneous electrical nerve stimulation has been shown to enhance perfusion of the skin, as shown by the laser Doppler technique,¹² but whether this also holds true for the musculature is less clear. The analgesic effects are thought to work by the gate control mechanisms and could well contribute to the effectiveness of this treatment in peripheral vascular disease.¹³ Epidural electrostimulation has also been reported to be helpful in patients with vascular disease, but the technique has the disadvantage of being invasive.¹⁴ At present the verdict must be that the place of electrical treatments in peripheral vascular disease needs further clinical assessment.

Yet another treatment is immersion of the patients in a bath

enriched with carbon dioxide.¹⁵ This causes hyperaemia of the skin, but this by no means indicates hyperaemia in the musculature beneath. There is also some evidence, however, that carbon dioxide might restore the flow characteristics of the blood towards normal.¹⁶ Recently we concluded a controlled clinical trial of 800 patients with cardiovascular disease showing that regular carbon dioxide baths lowered blood viscosity (unpublished results). External carbon dioxide also improves blood flow in the microcirculation of the skin.¹⁷ This treatment has, therefore, a rational basis, but clinical proof of effectiveness is still lacking.

Clearly, physical medicine has a place in treating peripheral vascular disease. Exercise is the most successful and cost effective treatment in patients with intermittent claudication. Other approaches such as electrotherapy and external carbon dioxide may seem worth trying when all else has failed, but they need to be investigated more thoroughly. There are some even more bizarre options—ultrasound, ultraviolet radiation, massage, and intermittent compression; but at present these lack both a rationale and proof of effectiveness.¹⁸⁻²⁰

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