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CHRONIC NON-A, NON-B HEPATITIS CARRIER STATE

Transmissible Agent Documented in One Patient over a Six-Year Period

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NON-A, non-B hepatitis is a major health problem, present in up to 89 per cent of patients with post-transfusion hepatitis[1] and 25 per cent of hospitalized patients with sporadic hepatitis.[2] Experimental transmission to chimpanzees of human non-A, non-B hepatitis[3-7] and passage of an agent of non-A, non-B hepatitis to additional chimpanzees[8] have demonstrated the cause to be a transmissible agent or agents and provided an animal model for further study of the disease. These studies and others have led to the development of experimental assays designed to detect antigen-antibody systems present in serum[9,10] and liver[11] associated with non-A, non-B hepatitis.

Evidence of a chronic carrier state, in which an agent of non-A, non-B hepatitis is present in a patient's blood for years, has been derived from retrospective analyses of serum from persons implicated in transmission of non-A, non-B hepatitis to recipients of their blood.[3,12] Our study was designed to determine the duration of infectivity during chronic asymptomatic non-A, non-B hepatitis by inoculating into chimpanzees four serum or plasma samples obtained over a six-year period from one patient; one of these samples was obtained when the patient's aminotransferase levels had temporarily returned to normal.

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Methods

Case Report

A 23-year-old man was admitted to the Veterans Administration Medical Center, Washington, D.C. in June 1972, with a diagnosis of aplastic anemia (Fig. 1). He was given transfusions of 31 units of packed red cells and 43 units of platelets over the next seven months. He was also treated with folic acid, pyridoxine, oxymetholone, prednisone, and a variety of antibiotics to treat recurrent abscesses. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels first became elevated in November 1972, reaching peak AST levels of 1250 IU per liter in January 1973 (with ALT 300 IU per liter), and peak ALT levels of 700 IU in February 1973. Bilirubin remained below 1.8 mg per deciliter. AST and ALT gradually decreased over the next five years, remaining between 40 and 53 IU per liter from 1976 to 1978. In October 1978 the patient's AST was 39 IU per liter, and ALT 45 IU per liter. The first entirely normal values (≤40 IU per liter) were noted in April 1979, when the AST was 30 IU per liter, and ALT 37 IU per liter, although both levels were again elevated in January, February, and March 1980. No symptoms of hepatitis were present at any time. A liver biopsy performed in March 1980 showed mild to moderate lymphocytic infiltration of the portal areas, with mild portal fibrosis. In only one portal area did the inflammation extend into the parenchyma. These findings were consistent with a diagnosis of chronic persistent hepatitis. The patient had two additional hospitalizations: one in 1974 for paroxysmal nocturnal hemoglobinuria, and one in 1975 for bone-marrow biopsy.

On March 15, 1973, a nurse caring for this patient in the outpatient department injured herself on a broken capillary pipette contaminated with the patient's blood (Fig. 2). On March 15 and April 12, she received intramuscular injections of 5 ml of immune serum globulin as a participant in a Veterans Administration Cooperative Study to evaluate prophylaxis against hepatitis after exposure. On April 25 she had abdominal pain and polyarthralgia, followed by anorexia on April 28. On April 30, seven weeks after the accidental inoculation, her AST and ALT, which had both been lower than 40 IU per liter when tested on the day of her accidental inoculation and two and four weeks later, were 338 IU per liter and 440 IU per liter, respectively. Her total bilirubin was 0.6 mg per deciliter. She continued to have malaise and intermittent anorexia and nausea until August 6, 1973, and her AST and ALT remained between 70 and 247 IU per liter until September 10, 1973, at which time they returned to normal. A percutaneous liver biopsy on August 2, 1973 (Week 20) revealed lobular disarray, acidophilic degeneration, acidophilic bodies, lymphocytic infiltration of the sinusoids, and evidence of parenchymal regeneration (anisonucleosis and binucleation); these findings were consistent with resolving acute hepatitis.

Both the patient and the nurse were diagnosed as having non-A,

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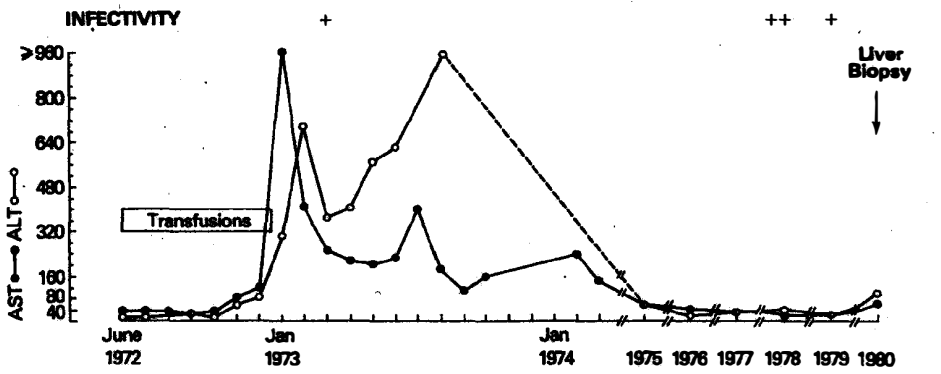

Figure 1. Data on a Patient with Asymptomatic Chronic Non-A, Non-B, Hepatitis.

Infectivity was detected at times indicated by plus signs and determined through experimental inoculation of chimpanzees with the patient's serum or plasma. AST denotes aspartate aminotransferase, and ALT alanine aminotransferase (normal, <40 IU per liter). Transfusions of 31 units of packed red cells and 43 units of platelets were given during the seven months indicated. Liver biopsy was performed at week indicated (see text).

non-B hepatitis because their serum samples were consistently negative for the markers of hepatitis B virus infection according to the most sensitive tests available (radioimmunoassays for hepatitis B surface antigen [HBsAg], for antibody to HBsAg, and for antibody to hepatitis B core antigen) and were negative or of constant titer when tested by immune adherence hemagglutination and radioimmunoassay for antibody to hepatitis A virus.

Inocula

Serum was obtained from the patient on March 15, 1973 (16 weeks after the onset of aminotransferase elevations and the day on which the nurse accidentally inoculated herself with a broken capillary pipette contaminated with his blood; Inoculum Ia). Serum was again obtained in February 1978 (5½ years after the onset of aminotransferase elevations; Inoculum Ib) and in October 1978 (six years after the onset of aminotransferase elevations; Inoculum Ic), and plasma was obtained in citrate-phosphate-dextrose in April 1979 (6½ years after the onset of aminotransferase elevations; Inoculum Id). Inoculations of chimpanzees were performed asynchronously over two years. Chimpanzees 922 and 930 were each inoculated intravenously with 1 ml of a 1:10 dilution of Inoculum Ia, as described elsewhere.[3] Chimpanzees 960 and 961 were each inoculated intravenously with 1 ml of a 1:10 dilution of Inoculum Ib. Chimpanzee 975 was inoculated intravenously with 1 ml of undiluted Inoculum Ic, and Chimpanzee 993 with 1 ml of undiluted Inoculum Id.

Chimpanzees

The six inoculated chimpanzees (*Pan troglodytes*) were born in a breeding colony in the United States (International Center of Environmental Safety, Alamogordo, N.M., FDA contract No. 223-77-1004), and at the start of the study they were 14 to 20 months old and weighed from 6 to 9 kg. The care and feeding of these chimpanzees have been described elsewhere.[3,13] There was little likelihood of prior exposure to hepatitis viruses; the infant chimpanzees, their parents, and their human caretakers were monitored regularly for abnormalities of AST and ALT, and for the presence of HBsAg, antibodies to HBsAg, and antibodies to hepatitis B core antigen. None of the chimpanzees except 993 had been inoculated with any other serum; 993 had been inoculated with a 1:1000 dilution of Inoculum Ia 16 weeks previously. This dilution was found to be noninfectious in this chimpanzee and two others.

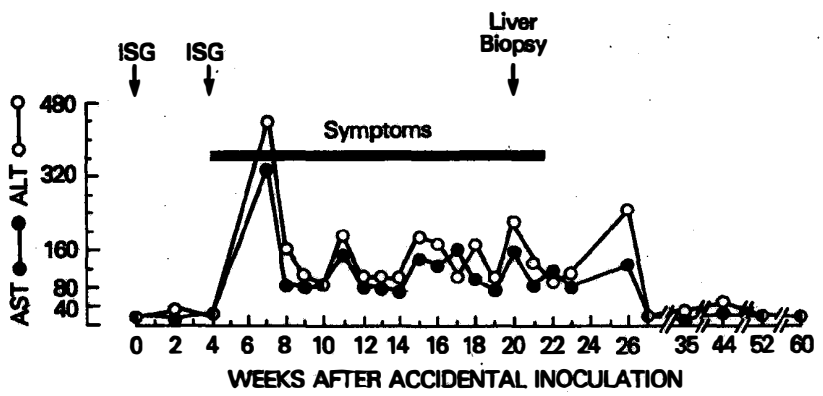

Figure 2. Data on Nurse Infected with Non-A, Non-B Hepatitis by Accidental Inoculation with Blood from Patient Shown in Figure 1.

ISG denotes inoculation of immune serum globulin, AST aspartate aminotransferase, and ALT alanine aminotransferase (normal, <40 IU per liter). Liver biopsy was performed at week indicated (see text). Bar denotes period during which symptoms were present.

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Serologic Studies and Liver Biopsies

Beginning four weeks before inoculation and continuing throughout the study, serum specimens from each of the six chimpanzees were tested weekly for AST and ALT (normal, <40 IU per liter)[14] and for isocitric dehydrogenase[15]; HBsAg[16] and antibodies to HBsAg[17] were tested weekly by means of radioimmunoassay, and antibodies to hepatitis B core antigen by means of complement fixation[18] and radioimmunoassay.[19] We tested selected serum samples for antibodies to hepatitis A virus by immune adherence hemagglutination[20] and radioimmunoassay,[21] for antibody to cytomegalovirus by radioimmunoassay,[22] and for antibodies to Epstein–Barr virus by immunofluorescence.[23] Serial serum samples from these chimpanzees were tested by means of counterelectrophoresis for an antigen-antibody system previously shown to be associated with non-A, non-B hepatitis.[9] Percutaneous liver biopsies were performed weekly, with anesthesia induced by cyclohexylamine, a drug with no known toxic effects on the liver. Biopsy specimens were stained with hematoxylin and eosin and assessed according to previously reported criteria.[3,13]

Table 1. Non-A, Non-B Hepatitis in Chimpanzees Inoculated with Serum or Plasma Obtained from a Chronically Infected Patient over Six Years.

Date Patient Serum Obtained	Recipient Chimpanzee No.	Weeks of Elevated AST/ALT*	Weeks of Abnormal Liver Specimen‡	Weeks of Associated Antigen§	Weeks of Associated Antibody§
3/73	922	2–17	9–14	4–9, 15	24+
	930	3–23	4–15,† 21	1–10,† 18	23+
2/78	960	2–19 †	8–10, 17	None	3+ †
	961	3–19 †	8, 9, 16, 17	None	16+ †
10/78	975	4–14 †	7, 17	None	7+ †
4/79	993	4, 13–16	4–8 †	4	6+ †

*AST denotes aspartate aminotransferase, and ALT alanine aminotransferase (normal, <40 IU per liter). Weeks indicated are calculated from date of inoculation.

†Normal values were occasionally detected during the period indicated.

‡Abnormal liver-biopsy specimens are defined in the text.

§Associated antigen and antibody denotes antigen-antibody system associated with non-A, non-B hepatitis detected by counterelectrophoresis, as described in the text.

Results

Each of the four samples of the patient's serum or plasma transmitted non-A, non-B hepatitis to the inoculated chimpanzees (Table 1). AST or ALT or both became elevated two to four weeks after inoculation, with peak AST levels of 55 to 306 IU per liter, and peak ALT levels of 92 to 227 IU per liter. Levels of isocitric dehydrogenase paralleled those of AST and ALT (data not shown). Histopathologic changes typical of hepatitis[3,8] were seen in weekly liver-biopsy specimens during the period of aminotransferase elevation. None of the six infected chimpanzees acquired HBsAg or antibodies to HBsAg, hepatitis B core antigen, hepatitis A virus, or cytomegalovirus; titers of antibodies to Epstein–Barr virus remained unchanged in all six chimpanzees. Although not all six chimpanzees were inoculated or infected simultaneously, uninoculated control chimpanzees were housed in the same facility throughout this study, and none of the controls had elevations in AST or ALT or abnormalities detected on liver biopsy.

An antigen associated with non-A, non-B hepatitis[9] was detected with counterelectrophoresis in chimpanzees 922, 930, and 993 at the time of AST and ALT elevations and histopathologic changes in liver-biopsy specimens (Table 1), but not in chimpanzees 960, 961, or 975. In all six chimpanzees the development of antibody to the non-A, non-B-hepatitis-associated antigen was detected by means of counterelectrophoresis.

Discussion

This study documents the persistence of an agent of non-A, non-B hepatitis in the blood of a patient for six years, even at a time when aminotransferase levels had temporarily returned to normal. Earlier studies suggesting the existence of chronic carriers of the non-A, non-B hepatitis agent or agents relied on retrospective analysis of prior episodes of transmission.[3,4,12] In this study, the similar incubation periods, the pattern of aminotransferase elevations, the serologic findings of an antigen-antibody system associated with non-A, non-B hepatitis,[9] and the histopathologic changes observed in biopsy specimens of the liver indicated that the same agent was present in this patient's blood throughout the six-year period.

Of great practical importance is the finding that plasma obtained from this asymptomatic patient, even after his AST and ALT levels had temporarily returned to normal, transmitted non-A, non-B hepatitis, documenting that this agent may persist even when sensitive indicators of liver damage are within the normal range. It has been suggested that elevated levels of AST or ALT may be used to identify blood donors who transmit non-A, non-B hepatitis to recipients of their blood.[24] Other studies have shown that blood from some donors with elevated AST or ALT may transmit non-A, non-B hepatitis to patients[25] or to experimentally inoculated chimpanzees[3] but that 80 per cent of blood donors implicated in transmitting non-A, non-B hepatitis have normal AST or ALT levels when tested retrospectively.[25] Our study confirms that blood from persons with non-A, non-B hepatitis can be infectious even when AST and ALT levels are normal.

An antigen-antibody system closely associated with non-A, non-B hepatitis and not with other types of hepatitis has been detected in serum by counterelectrophoresis.[9] This antigen-antibody system appears to be related to the hepatitis C antigen reported in the serum of some patients with transfusion-associated non-A, non-B hepatitis in Japan.[10] This antigen was present in the three samples from the patient in this study from whom sufficient serum was available for testing (Inocula Ib, Ic, and Id).[9] Antibody was present in each of four serum samples obtained during convalescence up to 6½ years after onset of infection from the nurse who was infected with non-A, non-B hepatitis by accidental inoculation with the blood of this patient[9]; (Tabor E, Seeff LB, Gerety RJ. Unpublished data). The antigen-antibody system was detected in all six chimpanzees inoculated with this patient's serum or plasma, including two described previously.[9] The appearance of the antibody in three

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of the chimpanzees (960, 961, and 975) while aminotransferase levels were elevated suggests that, if directed to a non-A, non-B hepatitis agent, the antibody does not immediately alter the course of the acute disease. Although many questions remain unanswered concerning this antigen-antibody system, detection of the antigen or the antibody in the blood of the patient, the nurse, and all six inoculated chimpanzees is further evidence that a single agent was responsible for all the infections in this study.

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LAW-MEDICINE NOTES

Multimillion-Dollar Verdict in Malpractice Case in Connecticut: A Complex Trial Record

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NEW ENGLAND has not been an area where very large verdicts have been common in medical malpractice cases. Widespread publicity was therefore to be expected when the Supreme Court of Connecticut recently upheld as proper and justified a jury verdict of $3.6 million.[1]

The defendant was a local general hospital with a psychiatric ward in which a 23-year-old psychotic patient harmed herself severely in a seclusion room, having been unattended for nearly four hours. The jury had not found negligence against the plaintiff's psychiatrist but returned its verdict only against the hospital. The defendant claimed that the verdict was obviously excessive, being four times as large as any ever returned in the state, and that "its size alone [was] indicative of prejudice, passion, bias, sympathy and total misunderstanding of the law and the facts on the part of the jury."

The trial judge had not only denied a motion to set aside the verdict as excessive but had also remarked that the evidence would have supported "a substantially higher verdict."

The trial judge's comments were certainly unusual in the relatively conservative judicial precincts of Connecticut. Most general discussion of the case will tend to note only the size of the verdict, not the circumstances of the case. As the entire, complex trial record is examined, however, the full picture of an aggravated, tragic situation is revealed. The features leading to a verdict so high can be summarized as follows: negligent failure to safeguard a very sick patient; a blatant effort by the hospital to rewrite, alter, and disguise the patient's record; serious neurologic injury and disability with conscious pain and suffering; and the requirement for lifelong medical and nursing care for a young plaintiff with a life expectancy of 41 years after the trial. Although it may not be part of the legal precedent for which the case will later be cited, one could also note the high technical quality of the plaintiff's expert witnesses in both medical and economic areas, the use of a 20-minute videotaped motion pic-