

to chronic liver disease whereas type A hepatitis seems to be a self-limited acute disease.²²

Fifth, we employed three tests of hepatocellular dysfunction, of which A.L.T. was the most consistent: it was the only enzyme raised in all five chimpanzees, and in four of them was the one showing greatest abnormality. G.G.T.P. had a very similar pattern to that of A.L.T. and in one chimpanzee reached a higher and more sustained level. However, in the two chimpanzees with the mildest A.L.T. increases, the G.G.T.P. remained normal. A.S.T. was the least reliable of the tests.

Lastly, the volume of inoculum did not seem to influence the severity of the ensuing disease; two of the three chimpanzees with severe disease had received less than 4 ml of infectious material, whereas the two with the mildest hepatitis received 75 ml. In future experiments, therefore, smaller inocula can be used. Similarly, the stage of human non-A, non-B hepatitis at which the blood was obtained did not seem to influence the disease course: one animal which received chronic-phase plasma had a response almost identical to those of the two animals receiving acute-phase serum.

We have shown that blood from human beings with apparent non-A, non-B hepatitis contains an agent capable of causing similar disease in chimpanzees. This animal model may now permit more definitive characterisation of non-A, non-B inocula, including infectivity titres; assessment of cross immunity to determine whether non-A, non-B hepatitis exists in more than one variety; detection of virus material in liver; and the use of such material in development of a serological test for non-A, non-B hepatitis.

We thank Delores Koziol, Max Shapiro, Dana James, and Lenita Hudson for expert technical assistance.

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TRANSMISSION OF NON-A, NON-B HEPATITIS FROM MAN TO CHIMPANZEE

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Summary Non-A, non-B hepatitis was transmitted to four colony-born chimpanzees by intravenous inoculation of human sera. Two chimpanzees were inoculated with serum from a patient with a clinical and serological diagnosis of chronic non-A, non-B hepatitis whose blood appeared to transmit this disease to a nurse following accidental needle-stick, and the other two chimpanzees were inoculated with serum from either of two former blood-donors whose HB_sAg-negative blood appeared to transmit clinically recognisable hepatitis, and who were found to have raised serum-aminotransferase levels 1½ and 5 years later. Serum-aminotransferase levels rose in all four chimpanzees, beginning 2–4 weeks after inoculation: peak alanine-aminotransferase values were 210 to 328 i.u./l. Evidence of hepatitis was present in liver biopsy specimens from all four chimpanzees, beginning 8–10 weeks after inoculation. None showed serological evidence of infection with hepatitis A virus, hepatitis B virus, cytomegalovirus, or Epstein-Barr virus.

Introduction

SINCE the discovery of hepatitis B surface antigen (HB_sAg)¹ and hepatitis A virus (H.A.V.),² hepatitis types A and B have become distinguishable by specific serological tests. A further type of hepatitis, in which no agent has yet been identified, has been designated "non-A, non-B hepatitis".^{3,4} Non-A, non-B hepatitis represents a high proportion of the cases of post-transfusion hepatitis in the United States, now that HB_sAg-positive donor blood is excluded.^{4,5} The association of a transmissible agent with this disease has been suggested by investigation of stored serum specimens taken in studies of volunteers in the 1950s.⁶ However, investigation of the disease has been hampered by the lack of a serum marker and of an animal model. We have tried to demonstrate a transmissible agent by inoculating human sera into young chimpanzees born in captivity.

Methods

Inocula

Inoculum I was obtained from a patient with aplastic anaemia at the Veterans Administration Hospital, Washington, D.C. Hepatitis developed after transfusion and aminotransferase levels remained high for more than 4 years. 6 weeks after the onset of his disease, a nurse caring for him injured herself with a broken capillary pipette contaminated with his blood.

She was entered into the Veterans Administration cooperative study to evaluate hepatitis-B immune globulin,⁷ and received conventional immune serum-globulin at the time of injury and again 4 weeks later. 6 weeks after injury she became anorexic with abdominal pain and arthralgias. Her serum-aminotransferase levels had been normal at weeks 0, 2, and 4, but at week 7 the aspartate aminotransferase (A.S.T.) was 336 I.U./l and the alanine aminotransferase (A.L.T.) was 440 I.U./l. Her aminotransferase levels remained raised until week 27, but hyperbilirubinemia was not detected. A liver biopsy specimen taken at week 20 showed lobular disarray, acidophilic degeneration, acidophilic bodies, lymphocytic infiltration of the sinusoids, and evidence of parenchymal regeneration (anisonucleosis and binucleation).

Both the patient and the nurse were diagnosed as having non-A, non-B hepatitis because the most sensitive available serological tests for hepatitis A and B virus infection—radioimmunoassay (R.I.A.) for HB_sAg and antibody to HB_sAg (anti-HB_s), and immune adherence haemagglutination (I.A.H.A.) for antibody to hepatitis A virus (anti-H.A.v.)—were negative. The patient's and nurse's sera were also negative for antibody to the hepatitis B core antigen (anti-HB_c) by complement fixation. Serum was drawn from the patient at the time of accidental inoculation of the nurse, and was stored for 4 years at -20°C. 24 hours before this study, a 1/10 dilution of this serum was prepared and stored at -70°C as inoculum I, for intravenous injection of 1 ml each into chimpanzees nos. 922 and 930.

Inocula II and III were obtained from former blood-donors placed on the American National Red Cross Donor Deferral Registry after their blood had been implicated in two separate cases of post-transfusion hepatitis in recipients of two-unit transfusions. Blood from these two donors was negative for HB_sAg, by counterelectrophoresis and R.I.A., respectively, at the time of donation, and hepatitis developed in the recipients. The recipient of blood from the donor of inoculum II was not tested for HB_sAg, while the recipient of blood from the donor of inoculum III remained negative for HB_sAg by R.I.A. Serum was obtained for further study from the donor of inoculum II 5 years later and from the donor of inoculum III 1½ years later, and was in each case found to be negative for HB_sAg, anti-HB_s, and anti-HB_c. These sera were selected for the present study when they were found to have A.S.T. levels of 92 I.U./l (inoculum II) and 73 I.U./l (inoculum III) and A.L.T. levels of 72 I.U./l (inoculum II) and 68 I.U./l (inoculum III). The sera were stored at -70°C until 24 hours before the study, when a 1/10 dilution of each was prepared and stored at -70°C as inocula II and III, for intravenous injection of 1 ml into chimpanzees nos. 918 and 919, respectively.

Chimpanzees

Four chimpanzees (*Pan troglodytes*) were studied; they were all born in a U.S. breeding colony, and at the start of the study they were about 19 months old and weighed from 7.5 to 9 kg. From birth to about 12 months of age they had been housed in a nursery and fed on infant formula; the care and feeding after age 12 months have been described elsewhere.⁸ There was little likelihood of exposure to hepatitis virus: the only potential sources were their human caretakers and infant chimpanzees, and these were tested monthly for A.S.T. and A.L.T.

For 12–19 months before the study began, monthly serum samples from each chimpanzee were tested and found negative for HB_sAg and anti-HB_s; for 3 months samples were also tested and found negative for anti-HB_c, hepatitis B e antigen (HB_eAg), and its antibody (anti-HB_e). One of the chimpanzees (no. 919) had recovered from H.A.v. infection induced experimentally 3 months before by inoculation with chimpanzee plasma containing H.A.v., and had an anti-H.A.v. titre of 1/1000. None of the other chimpanzees had detectable anti-H.A.v., and none had been previously inoculated with any other serum. All except the chimpanzee with prior experimen-

tal hepatitis A had A.S.T. and A.L.T. levels below 40 I.U./l throughout the period of observation.

After inoculation, serum specimens from all chimpanzees were tested weekly or twice-weekly for A.S.T. and A.L.T. with a biochromatic analyser (ABA-100, Abbott Laboratories, North Chicago, Illinois)⁹ (normal, 5–40 I.U./l), for isocitric dehydrogenase by the Sigma method¹⁰ (normal, 50–200 Sigma units), for HB_sAg by R.I.A. ('Ausria II', Abbott Laboratories),¹¹ for anti-HB_s by R.I.A. ('Ausab', Abbott Laboratories),¹² for anti-HB_c by complement fixation,^{13,14} and for HB_sAg and anti-HB_c by agar-gel diffusion.^{15,16} Selected serum samples were tested for anti-H.A.v. by immune adherence haemagglutination.^{17,18} Selected sera were tested for antibody to cytomegalovirus (anti-c.m.v.) by indirect immunofluorescence¹⁹ and radioimmunoassay,²⁰ and for antibody to the capsular antigen of the Epstein-Barr virus (anti-e.b.v.) by indirect immunofluorescence.²¹ Similar tests were performed on three uninoculated chimpanzees of the same age and size housed in the same facility.

Liver biopsy specimens were taken weekly, the animals being anaesthetised with cyclohexylamine, a drug with no known liver toxicity. The specimens, obtained with a 14 gauge Vim-Silverman needle, were stained with haematoxylin and eosin and were assessed by the criteria of Barker et al.⁸

Results

Biochemical and histological evidence of hepatitis developed in all four chimpanzees (fig. 1). Aminotransferases first became raised in week 2 (no. 922), 3 (no. 930), 4 (no. 918), and 3 (no. 919). The peak aminotransferase values (A.S.T. 87–138 I.U./l, A.L.T. 210–328 I.U./l) occurred between week 6½ and week 15. A.S.T. and A.L.T.

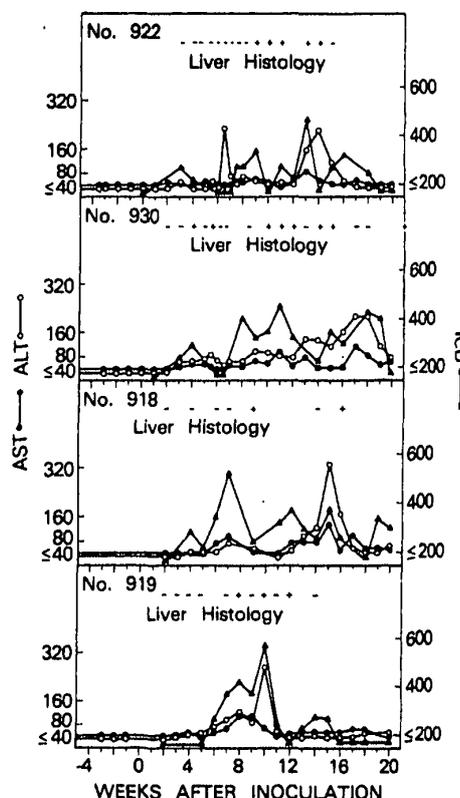


Fig. 1—Non-A, non-B Hepatitis in four chimpanzees. For abbreviations, see Methods. Liver histology: + = hepatitis, - = normal. Upper limits of normal for A.L.T., A.S.T., and I.C.D. are 40, 40, and 200 I.U./l.

returned to normal in chimpanzee no. 922 by week, 17, and to near-normal levels in chimpanzee no. 918 by week 18 and in chimpanzee no. 919 by week 20, but remained raised at week 20 in chimpanzee no. 930. Isocitric-dehydrogenase abnormalities were closely parallel to those of A.S.T. and A.L.T. in all four chimpanzees. None of the chimpanzees became clinically ill.

All four chimpanzees remained negative for HB_sAg, anti-HB_s, anti-HB_e, HB_eAg, and anti-HB_e. Chimpanzees nos. 922, 930, and 918 remained negative for anti-H.A.V. and the preinoculation anti-H.A.V. titre of 1/1000 in chimpanzee no. 919 did not change. Chimpanzees nos. 930, 918, and 919 remained negative for anti-C.M.V., while the preinoculation anti-C.M.V. titre of 1/30 in chimpanzee no. 922 did not change. The preinoculation anti-E.B.V. titres of 1/320 did not change in any of the chimpanzees.

All weekly biopsy specimens were read under code by three independent observers. They were graded on light microscopic examination either as negative (no changes or minimal changes) or as positive (fig. 1). Various abnormalities were noted in the biopsy specimens classed as positive (fig. 2). These included the parenchymal abnormalities of lobular disarray, smudging of cell outlines, acidophilic degeneration and frank acidophilic bodies, and focal necrosis; prominent lymphocytic infiltration involving principally the sinusoids, giving a "beaded" appearance, but with the infiltration involving also the portal areas in some of the specimens (nos. 922, 930, and 919); and marked hyperplasia of the Kupffer cells. Nuclear abnormalities were also noted in the livers of all four chimpanzees during the time enzymes were raised, but these specimens were designated positive only if inflammation was also present. The nuclear changes consisted of enlarged nuclei with peripheral displacement of the chromatin, but with retention of purplish-stained centrally located nucleoli, giving a "bull's-eye" appearance. Multinucleated hepatocytes, sometimes with as many as six nuclei per cell, were commonly seen.

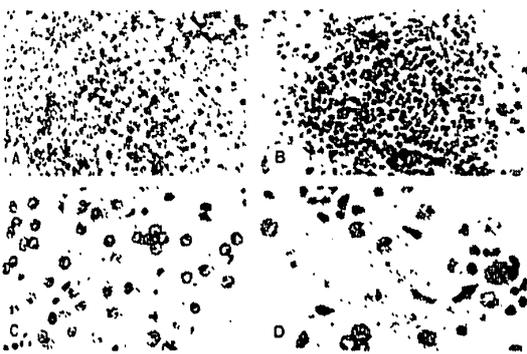


Fig. 2—Liver biopsy specimens from nurse accidentally inoculated by inoculum I (A) and chimpanzees no. 922 (B) and no. 930 (C, D) which were inoculated with inoculum I.

A. Lobular disarray, acidophilic degeneration and acidophilic bodies, lymphocytic inflammation in sinusoids, and evidence of regeneration (anisonucleosis and binucleation). $\times 290$.

B. Portal area showing profusion of lymphocytes extending into surrounding sinusoids. $\times 350$.

C. Multinucleated hepatocytes, enlarged nuclei with centrally located nucleoli ("bull's-eye" nuclei), and hyperplastic Kupffer cells. $\times 550$.

D. Sinusoidal inflammation hyperplastic Kupffer cells, multinucleated hepatocytes, and acidophilic body (arrow). $\times 750$.

On breaking of the code, the positive biopsy specimens proved to have been obtained when liver enzymes were raised.

Discussion

We have shown that non-A, non-B hepatitis is transmissible to young chimpanzees by sera from human beings whose blood had transmitted the disease to other human beings. Previous attempts at transmission to chimpanzees, by us and by other investigators, may have failed because the animals were older and possibly immune, or because the inocula were not infective.

The disease courses in our animals were strikingly similar, despite the different sources of inoculum. The time to the first rise in liver enzymes was 2–4 weeks, in contrast to the 7-week incubation period in the nurse. This may be related to use of immune serum-globulin in the nurse, differences in inoculum dose, or differences in host immune responses.

This study strongly supports the existence of chronic infection in non-A, non-B hepatitis in man, as suggested lately by Hoofnagle et al.⁶ The patient whose serum was used for inoculum I had transmitted non-A, non-B hepatitis to a nurse early in his 4-year course of non-A, non-B hepatitis. Inocula II and III were from former blood-donors with raised liver enzymes, whose blood was implicated in the transmission of an undetermined type of hepatitis 5 years before (inoculum II) and non-B hepatitis 1½ years before (inoculum III). Both cases were classified as non-A, non-B hepatitis because neither donor had any marker for hepatitis B and because transmission of hepatitis A by blood-transfusion is extremely rare.³

The prospect for further developments in the understanding and control of non-A, non-B hepatitis will be enhanced by this animal model. Serial passage of the infectious agent or agents, the development of titred infectious inocula, further study of biopsy tissue and serum, and cross-challenge and reinfection studies will be needed to establish the number of infectious agents involved and to determine whether active immunisation against infection is possible.

We thank Mr A. J. Shawver, Mr D. Gilbert, Mr J. Thiel, and Mr E. Rollins for technical assistance. This study was supported in part by U.S. Food and Drug Administration contracts no. 223-74-1103 and no. 223-73-1207.

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