TRANSMISSION OF NON-A, NON-B HEPATITIS AGENT TO CHIMPANZEES FROM PATIENTS OF EPIDEMIC HEPATITIS

Kenji ABE¹,², Toshio SHIKATA², Kiyoshi FUJISAWA³, Masayoshi YAMAUCHI³, Hiroshi MATSUSHITA⁴, Hiroshi SUZUKI⁵ and Toshitsugu ODA⁶

¹Department of Pathology, National Institute of Health, Gakuen, Musashimurayama-shi, Tokyo 190-12, ²Department of Pathology, Nihon University School of Medicine, Tokyo 173, ³Department of Medicine, Jikei University School of Medicine, Tokyo 105, ⁴Department of Public Health, Hamamatsu Medical College, Shizuoka 430, ⁵Department of Medicine, Yamanashi Medical College, Shizuoka 430, and ⁶National Medical Center Hospital, Tokyo 160

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SUMMARY: Two chimpanzees were inoculated intravenously with acute-phase sera obtained from two patients with epidemic hepatitis. They developed histopathologically confirmed hepatitis. Electron microscopic examination of the liver showed peculiar cytoplasmic tubular structures in the hepatocytes. These ultrastructural findings were similar to those described for the livers of chimpanzees inoculated with the F strain of non-A, non-B hepatitis agent derived from a posttransfusion hepatitis case. The chimpanzee that had recovered from hepatitis caused by the F strain of non-A, non-B hepatitis agent was re-challenged with the serum from one of the patients. The chimpanzee developed neither clinical signs nor histological changes of hepatitis. These results suggested that non-A, non-B hepatitis agent was involved not only in post-transfusion hepatitis but also in epidemic hepatitis.

All requests for reprints should be sent to Kenji Abe, Department of Pathology, National Institute of Health, 4-7-1, Gakuen, Musashimurayama-shi, Tokyo 190-12.

阿部賢治(国立予防衛生研究所病理部)
志方俊夫(日本大学医学部第1病理学教室 板橋区大谷口上町30-1)
藤沢 洋・山内真義(東京慈恵会医科大学第1内科学教室 港区西新橋3-25-8)
松下 寛(浜松医科大学公衆衛生学教室 浜松市半田町3600)
鈴木 宏(山梨医科大学第1内科学教室 中巨摩郡玉穂町下河東1110)
織田敏次(国立病院医療センター 新宿区戸山1-21-1)
INTRODUCTION

Non-A, non-B (NANB) hepatitis is a disease of presumed viral etiology that is frequently associated with transfusion of blood or blood products. The fact that NANB hepatitis is caused by transmissible agent(s) has been repeatedly confirmed by experimental infection in chimpanzees (1,2). On the other hand, sporadic and epidemic human cases of NANB hepatitis have also been reported (3-5). An outbreak of epidemic hepatitis occurred in 1980 and 1981 in Shimizu City, Shizuoka. One hundred and forty-one hepatitis cases were reported in Shimizu City. Epidemiological and clinical studies have been reported by Yamauchi and his co-workers (6). However, the route of infection is yet unknown. We have tried to isolate an agent transmissible to chimpanzees from cases of epidemic hepatitis.

MATERIALS AND METHODS

Transmission study: Two chimpanzees (No. 54 and No. 59) were used. Sera obtained from two patients of epidemic hepatitis in an acute phase were inoculated intravenously in 2-ml doses to the chimpanzees (Table I). After inoculation, blood was taken twice a week for biochemical liver function tests. Glutamate pyruvate transaminase (GPT) was determined with commercially available manual colorimetric kits (Marco Pharmaceutical Co., Nagoya), and gamma glutamyl transpeptidase (GGTP) with commercially available gamma-glutamyl-alpha-naphthylamide kits (Marco Pharmaceutical Co., Nagoya). Mean values for normal chimpanzees in our laboratory are 25 KU (SD = 4.5) for GPT, and 5.6 mu/ml (SD = 1.1) for GGTP. Liver needle biopsy specimens were taken twice a week and divided into two portions. One portion of the liver tissue was fixed in buffered

<table>
<thead>
<tr>
<th>Case (age/sex)</th>
<th>Clinical diagnosis</th>
<th>Peak GOT/GPT (mIU)</th>
<th>Inoculum</th>
<th>Chimp. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (44y/male)</td>
<td>Acute non-A,non-B hepatitis</td>
<td>773/886</td>
<td>2 ml serum</td>
<td>54</td>
</tr>
<tr>
<td>2 (30y/male)</td>
<td>Acute non-A,non-B hepatitis</td>
<td>573/861</td>
<td>2 ml serum</td>
<td>59</td>
</tr>
</tbody>
</table>
formalin for light microscopic examination. The remaining portion was fixed in 2.5% glutaraldehyde, followed by post-fixation in 1% osmium tetroxide, dehydrated in graded ethanols, and embedded in epoxy resin. Ultra-thin sections stained with lead citrate and uranyl acetate were examined with an electron microscope (JEM 100CX, JEOL Ltd., Tokyo). Liver biopsy specimens obtained before inoculation showed no pathological change in either of the two chimpanzees.

Cross-challenge study: One chimpanzee (No. 48) had been inoculated with acute phase serum from a chimpanzee (the second passage material of the strain F of NANB hepatitis agent in chimpanzees). The chimpanzee's serum was kindly provided by Dr. R. H. Purcell, N. I. H., USA (7). The chimpanzee developed biochemical and ultrastructural evidences of NANB hepatitis. After recovery from hepatitis, he was re-challenged with the same inoculum, but developed neither clinical signs nor ultrastructural evidences of hepatitis. He was subsequently cross-challenged by inoculation with the same serum as that given to No. 54.

RESULTS

No. 54 showed a slightly elevated level of GPT (above 30 KU) 13 to 22 weeks, and of GGTP (above 7 μu/ml) 4 to 30 weeks after inoculation (Fig. 1). Liver biopsy specimens showed mild lymphocytic infiltration in portal areas 7 to 8 weeks after inoculation.

No. 59 showed a slightly elevated level of GGTP (above 7 μu/ml) 4 to 26 weeks after inoculation (Fig. 1). Liver biopsy specimens showed severe lymphocytic and plasma cell infiltration in portal areas and hyperplasia of Kupffer cells during the period from 4 to 16 weeks after inoculation (Fig. 2).

Electron microscopic examinations of the livers of both chimpanzees revealed peculiar cytoplasmic tubular structures in hepatocytes from the 4th week (Fig. 3). They were electron-dense circular structures in the cross section and consisted of two parallel walls when cut longitudinally. The walls of the tubules were composed of double-unit membranes. The total thickness of the wall was about 20 nm. Occasionally, cross-striation was observed in the wall. Furthermore, other ultrastructural changes were observed in hepatocytes: convoluted smooth endoplasmic reticulum with electron-dense substance entrapped between paired cisternae (Fig. 4), sponge-like inclusions composed of a dense matrix and irregularly arranged membranes (Fig. 5), and aggregations of microtubular structures with a stacked-disk appearance, measuring 20 nm in diameter (Fig. 6). The microtubular structures had cross bandings when cut longitudinally.

There was no serological evidence for type A or type B hepatitis.

No. 48 developed neither clinical signs nor ultrastructural evidences for hepatitis after the cross-challenge inoculation (Fig. 7).
Fig. 1. Clinical course of chimpanzees inoculated with the sera from patients with epidemic hepatitis in Shimizu City.

Liver histopathology: + = hepatitis, − = no abnormal findings.

Ultrastructural change: + = positive and − = negative for tubular structures of hepatocytes.
Fig. 2. Histopathological findings with a liver biopsy specimen obtained from chimpanzee No. 59, 10 weeks after inoculation. Infiltration of numerous mononuclear cells in the portal area, mild lymphocytosis in sinusoids, and Kupffer cell hyperplasia, but no spill over of portal infiltrate and necrotizing inflammatory reaction. Hematoxylin and eosin, ×100.

Figs. 3-6. Ultrastructural findings with a liver biopsy specimens obtained from chimpanzee No. 54, 10 weeks after inoculation. Fig. 3. Peculiar cytoplasmic tubular structures in the endoplasmic reticulum of hepatocyte. ×40,200.
Fig. 4. Convoluted and curved double-membraned structures in the cytoplasm of hepatocyte. $\times 34,200$.

Fig. 5. Sponge-like inclusion with high electron density within an area of proliferated smooth endoplasmic reticulum of hepatocyte. Curved double-membraned structures surrounding this inclusion. $\times 28,000$. 
Fig. 6 Aggregation of microtubular structures measuring 20 nm in diameter with cross banding, which suggests that the microtubular structures consist of multiple stacked disk. ×60,000.

Fig. 7. Clinical course of chimpanzee No. 48 that was initially infected with the F strain NANB agent and then re-challenged with the same inoculum. Subsequently, he was cross-challenged by inoculation of the same serum material as that given to chimpanzee No. 54. Thin arrows indicate initial and re-challenge inoculations of the F-strain of NANB hepatitis agent. The thick arrow indicates cross-challenge inoculation.

Ultrastructural change: Closed squares positive and open squares represent negative for tubular structures of hepatocytes.
DISCUSSION

NANB hepatitis was first recognized as a disease associated with transfusion of blood and the administration of blood products. However, about half of all patients with sporadic acute hepatitis were attributed to NANB hepatitis in Japan (8). Epidemic outbreaks of NANB hepatitis were reported also in India. It was suspected that it might be caused by water-borne infection (3). An epidemic outbreak of NANB hepatitis in Shimizu City was observed in 1980 and 1981. Epidemiological study excluded water-borne infection, because there was no relationship between the source of water and the incidence of hepatitis (9). No case had received blood transfusion or any surgical operation before the onset of hepatitis. The route of infection is yet unknown.

We successfully transmitted hepatitis to chimpanzees. They were given acute-phase sera obtained from patients with epidemic hepatitis in Shimizu City. They developed hepatitis biochemically and histopathologically and electron microscopic examinations of the livers revealed cytoplasmic tubular structures in hepatocytes. These ultrastructural findings were similar to those described for the livers of chimpanzees inoculated with the F strain of NANB hepatitis agent derived from a post-transfusion hepatitis case (10). Furthermore, after cross-challenge, No. 48 developed neither transaminase elevation nor ultrastructural changes of hepatocyte, suggesting that the infectious agent detected in the present study was immunologically related to the F strain of NANB hepatitis agent. Therefore, the outbreak of epidemic hepatitis in Shimizu City might have been caused by a NANB hepatitis agent similar to the F strain. These results suggest that NANB hepatitis agent is closely associated with not only post-transfusion hepatitis but also epidemic hepatitis.

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