Lack of Detectable Reverse Transcriptase Activity in Human and Chimpanzee Sera with a High Infectivity for Non-A, Non-B Hepatitis

By YUKIO ITOH, SHIGENORI IWAKIRI, KOHJI KITAJIMA, TOHRU GOTANDA, MICHIKO MIYAKI, YUZO MIYAKAWA AND MAKOTO MAYUMI

1Hepatitis Division and 2Department of Biochemistry, The Tokyo Metropolitan Institute of Medical Science, Bunkyo-Ku, Tokyo 113, 3Institute of Immunology, Bunkyo-Ku, Tokyo 113 and 4Immunology Division, Jichi Medical School, Minamikawachi-Machi, Tochigi-Ken 329-04, Japan

(Accepted 6 January 1986)

SUMMARY

A serum sample from a patient with hepatitis and samples from two experimentally infected chimpanzees, all with a high infectivity for non-A, non-B hepatitis, were tested for reverse transcriptase. Biopsy confirmed that the hepatocytes of the chimpanzees that received these sera contained the characteristic tubular structures associated with non-A, non-B hepatitis. None of these three sera revealed detectable enzyme activity. We have not been able to confirm the association of reverse transcriptase activity with non-A, non-B hepatitis reported recently.

Seto and her colleagues (1984) have reported detection of reverse transcriptase (RT) activity in four sera and two plasma-derived products with a confirmed infectivity for non-A, non-B hepatitis (NANBH), as well as in all of 12 serum samples obtained during the acute phase from NANBH patients, and suggested that the NANBH agent(s) may be a retrovirus or retrovirus-like. Their report aroused immediate interest (Anonymous, 1984; Pouletty et al., 1985), because all previous attempts at the in vitro identification of materials infectious for NANBH had been unsuccessful (Dienstag, 1983).

We have studied three serum samples containing NANBH agent. Serum 1 was obtained from a patient with post-transfusion NANBH, diagnosed by serological exclusion of type A or type B hepatitis. Serum 2 was an acute phase serum from a chimpanzee which had contracted NANBH after inoculation with a preparation of fibrinogen that had been implicated in NANBH (Yoshizawa et al., 1980). Serum 3 was an acute phase serum of a chimpanzee which had developed NANBH after receiving serum from a healthy blood donor with an elevated level of glutamic pyruvic transaminase (Yoshizawa et al., 1982).

All three serum samples were highly infectious, with chimpanzee infectious doses (CID) of more than 10^2 per ml, causing a type of NANBH characterized by cytoplasmic tubular structures (Fig. 1). RT activity was tested by the standard method (Temin & Mizutani, 1974) with a slight modification. Samples (100 μl) were applied to a Beckman SW60 tube (capacity 4 ml) containing 3 ml 35% glycerol in Tris-HCl buffer (0.5 M, pH 7.0), and overlaid with 0.4 ml of Tris-HCl buffer. The tube was centrifuged at 94000 g for 90 min. The pellet was suspended in 40 μl 0.25% NP40 in Tris-HCl buffer. To the suspension was added 40 μl of a solution consisting of 75 mM-Tris·HCl pH 8.3, 100 mM-KCl, 10 mM-MgCl2, 25 mM-dithiothreitol, 5 μg actinomycin D, 100 μM each of unlabelled dATP, dCTP and dGTP, as well as 1 μM-[3H]dUTP (sp. act. 30 Ci/mmol) and 40 μM-poly(rA)-poly(dT)12–18. The mixture was incubated at 37°C for 60 min, and spotted onto a disc of Whatman DE81 filter paper. It was washed with 0.5 M-Na2HPO4 and then with water. The paper disc was dried, and the radioactivity was determined by liquid scintillation. The method was essentially the same as that of Seto et al. (1984) modified by the use...
Fig. 1. A hepatocyte from a chimpanzee subjected to biopsy 5 weeks after inoculation with serum 1 obtained from a patient with non-A, non-B hepatitis, diluted 1:1000. Numerous tubular structures that appear circular in cross-section were observed. Chimpanzees inoculated with the other two serum samples containing non-A, non-B hepatitis agent (Table 1) showed similar histological changes also. Bar marker represents 1 μm.

of filter paper (Maniatis et al., 1982) in place of glass fibre filters, in an attempt to reduce the background binding, and a 25-fold increase in the specific activity of radiolabelled nucleotide to increase the sensitivity.

All of the tested sera failed to exhibit any detectable RT activity, even though a positive control containing Kirsten murine sarcoma virus displayed an activity reasonable for the amount of virus present (Table 1).

The reason for the absence of RT activity in materials with a high infectivity for NANBH has not been established. There are two kinds of NANBH. One is transmitted by the faecal–oral route like type A hepatitis, and the other is transmitted by blood or plasma-derived products like type B hepatitis. At least two NANBH agents that are transmitted parenterally have been recognized by re-infection and cross-challenge studies in chimpanzees (Hollinger et al., 1980); one of these is associated with tubular structures in chimpanzee hepatocytes (Yoshizawa et al., 1981).

The possibility that Seto’s group and ours have dealt with different NANBH agents is remote. They also studied an agent that induces characteristic tubular structures in chimpanzee hepatocytes (Shimizu et al., 1979; Jackson et al., 1979). It might have been expected that there would have been at least some overlap between the infectious agent(s) contained in their specimens and ours. Our results are in agreement with those of Hallam (1985) who failed to detect RT activity in any of 20 serum samples from patients with NANBH, including four from those with blood-transmitted NANBH, diagnosed by serological exclusion of viruses known to induce hepatitis.

In view of their potential profound impact, the findings of Seto et al. should be treated with caution. Further tests for RT in materials with a known infectivity for NANBH in other
Table 1. Reverse transcriptase activity in human and chimpanzee sera with infectivity for non-A, non-B hepatitis

<table>
<thead>
<tr>
<th>Material</th>
<th>Species</th>
<th>CID/ml (c.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>Human</td>
<td>&gt; 10³ 39</td>
</tr>
<tr>
<td>Serum 2</td>
<td>Chimp</td>
<td>&gt; 10² 51</td>
</tr>
<tr>
<td>Serum 3</td>
<td>Chimp</td>
<td>&gt; 10³ 36</td>
</tr>
<tr>
<td>Normal serum</td>
<td>Human</td>
<td>0 36</td>
</tr>
<tr>
<td>Normal serum</td>
<td>Chimp</td>
<td>0 46</td>
</tr>
<tr>
<td>Buffer</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td>Kirsten murine sarcoma virus</td>
<td></td>
<td>5 × 10⁴ 8936</td>
</tr>
</tbody>
</table>

...laboratories are required. An attempt to correlate RT activity with infectivity, in terms of CID, has not been made. This is an essential step in evaluating the possible association of RT activity with NANB hepatitis agent. Until independent confirmation is available, the claim for RT activity in sera infectious for NANB hepatitis remains unestablished.

REFERENCES


(Received 26 November 1985)