Evidence of a Hepadna Virus Infection in Manchurian Chipmunks†

Byung Chul Yoo* and Chung Yong Kim**

Liver Research Institute, College of Medicine, Seoul National University***
Department of Internal Medicine, College of Medicine, Chungang University*  
Department of Medicine, College of Medicine, Seoul National University**

= Abstract = In attempting to find a new host of hepatitis virus, sera of Manchurian chipmunks (Tamias sibiricus asiaticus) were tested for serologic markers of hepatitis B virus and virus-like particles.

Of 47 tested animals, HBsAg reactivities were found in sera of 6 animals and anti-HBs in 7 animals by radioimmunoassay. The HBsAg-positive sera were examined by electron microscopy. Numerous spherical particles 21 nm in diameter and many spherical double-shelled particles 41 nm in diameter, which look similar to Dane particles, were observed in all the HBsAg-positive sera.

The amount of 41 nm particles seems to correlate with the titer of HBsAg reactivities. Histologic examination of liver tissues from the HBsAg and particles-positive animals showed no evidence of liver disease.

The detection of HBsAg reactivities and particles similar in appearance to the particles of hepatitis B virus in sera of Manchurian chipmunks strongly suggests infection of a hepatitis virus in these animals.

Key Words: Hepadna virus, HBsAg, Virus particle, Manchurian chipmunk

INTRODUCTION

When hepatitis B virus (HBV) was first identified and characterized, it was found to be distinct from all other known viruses. Uniques characteristics included its ultrastructure, antigenic makeup, size and structure of DNA, virion DNA polymerase, liver tropism, and features of persistent infection. The virion of HBV (Dane particle) is a double-layered spherical particle approximately 42 nm in diameter (Dane et al. 1970) consisting of a lipid-containing outer shell bearing hepatitis B surface antigen (HBsAg) and an inner spherical core or nucleocapsid approximately 27 nm in diameter. The viral core contains the hepatitis B core antigen (HBCag) (Almeida et al. 1971), the viral DNA (Robinson et al. 1974), a DNA polymerase activity (Kaplan et al. 1973), and hepatitis Be antigen (HBeAg) in a cryptic form (Takahashi et al. 1979).

The viral genome is a circular partially double-stranded DNA molecule with a length of approximately 3200 base pairs (Robinson et al. 1974), which has a single stranded region variable in length from 15% to 50% of the circle length in different molecules. A DNA polymerase activity in the virion repairs the single stranded region in the viral DNA to make fully double stranded molecule (Landers et al. 1977; Summers et al. 1975).

Infection with HBV is accompanied by presence in the blood of Dane particles and much higher concentrations of small spherical particles 22 nm in diameter and long filamentous particles with 22 nm width and variable length consisting of excess viral coat protein materials bearing HBsAg (Bayer et al. 1968; Dane et al. 1970).

Persistent infection with HBV is common, continues for many years, and may be associated with chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Beasley et al. 1981; Popper et al. 1982; Redecker 1975; Tong et al. 1981). The host range

†This work was supported in part by the grant from Seoul National University Hospital and from the Liver Research Foundation of Korea.
of HBV is confined to humans, the natural host, and a few other higher primates such as chimpanzees (Barker et al. 1972). The narrow host range and the lack of a tissue culture system for HBV replication hampered progress in studying viral replication and host response.

Recently, however, three animal viruses named woodchuck hepatitis virus (WHV), ground squirrel hepatitis virus (GSHV), and duck hepatitis B virus (DHBV), that have biological features very similar to HBV, have been found in woodchucks (Summers et al. 1978), beechey ground squirrels (Marion et al. 1980), and Pekin ducks (Mason et al. 1980). These viruses have many features of HBV including similar ultrastructure, partially double stranded DNA of similar size, a virion DNA polymerase activity, surface and core antigens cross-reacting with HBSAg and HBCAg, liver tropism, and common occurrence of persistent infection.

HBV and the three related viruses of lower animals are now called the hepadna viridae (Robinson 1980), and such animal viruses have been valuable in defining the characteristics of this family of viruses and may be useful models for the replication and pathogenesis of HBV.

In this report, we describe the evidence of a hepadna virus infection in Manchurian chipmunks inhabiting Korea.

MATERIALS AND METHODS

1. Animals

The animals used in this study were apparently healthy Manchurian chipmunks (Tamias sibiricus asiaticus) weighing approximately 80 gm captured from fields in Kyunggido and Kangwondo provinces of Korea. Sera were obtained from all animals by cardiac puncture and extracted liver tissues were fixed in 10% neutral formalin.

2. Detection of HBV markers

Each serum was tested for HBSAg, anti-HBs, and anti-HBc by radioimmunoassay using commercial kits (Abbott laboratories, North Chicago, III.)

3. Detection of particles by electron microscope

To test for the presence of particles similar to HBV in chipmunks sera, sera were pelleted and examined by electron microscope. Each serum sample was centrifuged at 10,000 rpm for 10 min to remove precipitated protein and other debris; 200 μl of each supernatant diluted with 800 μl of buffer was layered over 11 ml gradient of 10-20% (wt/vol) sucrose containing 10 mM Tris – HCL pH 7.4, 0.1M NaCl, and 5 mM EDTA. After centrifugation for 13 hr at 35,000 rpm in a Spinco SW 40.1 rotor at 20°C, the supernatant was thoroughly removed by aspiration, and the pellet was suspended in 100 μl of the buffer. The suspension was spotted on carbon-coated grid and the material was stained with 1% phosphotungstic acid and examined by electron microscope.

Histologic examination

Liver tissue fixed in 10% neutral formalin were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

RESULTS

1. Detection of HBV markers

Of 47 tested animals, HBSAg reactivities were found in sera of 6 animals and anti-HBs in 7 animals by radioimmunoassay. Anti-HBc was not detected in any case (Table 1). No HBSAg-positive serum produced more than 10-fold amount of 125I-labeled anti-HBs binding than that of HBSAg-negative control serum.

2. Detection of particles by electron microscope

All the HBSAg-positive sera contained the particles shown in Fig. 1. The most abundant form in all the HBSAg-positive sera was a small spherical particle 20-22 nm (Mean 21 nm) in diameter. All the HBSAg-positive sera also contained double-layered spherical particles 39-42 nm (mean 41 nm) in diameter with an outer shell and an inner spherical core, similar to the Dane particles of HBV. But filamentous form of particle was not observed in any of the HBSAg-positive serum. The amount of the double-layered particles appears to correlate with the titer of HBSAg reactivities (Table 2). No such particles were found in HBSAg-negative sera including anti-HBs-positive ones.

3. Histologic examination

Histologic exam of liver tissues from chipmunks containing HBSAg reactivities and virus-like particles in their sera reavealed no evidence of cell necro-

<table>
<thead>
<tr>
<th>Table 1. Testing of sera of Chipmunks for HBV markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBSAg</td>
</tr>
<tr>
<td>anti-HBc</td>
</tr>
<tr>
<td>anti-HBs</td>
</tr>
<tr>
<td><strong>Total 47</strong></td>
</tr>
</tbody>
</table>
Fig. 1. Electron micrograph of particles in HBsAg-reactive sera of chipmunks. (A: animals no.41 B: animal no.33)
Large arrow; Dane particles-like spherical double shelled particles (41 nm)
Small arrow; Small spherical particles (21 nm)
Bar represents 100 nm

Fig. 2. Hematoxylin/Eosin-stained sections of liver from chipmunk, which contains HBsAg reactivity and virus-like particles in serum

...strongly suggests a hepatitis B infection, since WHV and GSHV is known to have the surface antigens cross-reacting with HBsAg (Werner et al. 1979; Gerlich et al. 1980; Feitelson et al. 1981), and no other known virus has such a cross-reacting antigen (Purcell et al. 1970). Also the double-layered spherical particles 41 nm in diameter similar in appearance to hepatitis B virions (Dane particles) and higher concentration of smaller spherical particles 21 nm in diameter similar to surface antigen particles of HBV have been found in all the HBsAg-positive sera. The detection of HBsAg reactivities and particles similar to those of HBV is a strong evidence of a hepatitis virus infection, and other characteristics of the hepatitis viruses such as size and structure of the viral DNA, a DNA polymerase activity, liver tropism and persistent infection should be clarified. We have not been able to perform an analysis on the viral DNA due to shortage of the serum samples, because only less than 1 ml of serum could be obtained by cardiac puncture in these animals.

Although Manchurian chipmunks belong to the

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>HBsAg RIA S/N ratio</th>
<th>21 nm particles</th>
<th>41 nm particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>2.4</td>
<td>numerous</td>
<td>rare</td>
</tr>
<tr>
<td>14</td>
<td>2.3</td>
<td>numerous</td>
<td>rare</td>
</tr>
<tr>
<td>33</td>
<td>4.4</td>
<td>numerous</td>
<td>many</td>
</tr>
<tr>
<td>41</td>
<td>7.0</td>
<td>numerous</td>
<td>many</td>
</tr>
<tr>
<td>42</td>
<td>8.9</td>
<td>numerous</td>
<td>many</td>
</tr>
<tr>
<td>43</td>
<td>5.8</td>
<td>numerous</td>
<td>many</td>
</tr>
</tbody>
</table>
Sciuridae family like woodchucks and ground squirrels, the virus-like particles found in sera of chimpanzees are similar but not identical to those associated with WHV and GSHV. The 41 nm double-layered particles are somewhat smaller than virions of WHV(45 nm) and GSHV(47 nm), and filamentous form of particle was not found in contrast to high concentrations of filamentous particles in sera associated with WHV and GSHV infection. To clarify the relatedness and difference with WHV and GSHV, direct comparison of antigenic proteins and DNA would be required.

In the woodchucks, persistent infections with WHV are generally accompanied by chronic active hepatitis and animals with this aggressive disease have shown a high incidence of hepatocellular carcinoma (Frommel et al. 1984; Millman et al. 1984; Popper et al. 1981; Summers et al. 1978). In contrast, the infections of GSHV in ground squirrels (Marion et al. 1983) and DHBV in Pekin ducks are essentially silent in studies in the USA, although in some provinces of China DHBV infections were associated with chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Omata et al. 1983). Histologic exam of liver tissues from the chimpanzees containing HBsAg and virus-like particles in sera revealed no evidence of liver disease, so these animals were considered to be healthy carriers. But long-term follow-up exam of chimpanzees bearing virus-like particles would be required to clarify the existence of associated liver disease.

REFERENCES
Almeida JD, Rubenstein D, Stott EJ. New antigen-antibody system in Australia-antigen positive hepatitis. Lancet 1971;ii:1225-1227
Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. Lancet 1970, i:695-698
Landers T, Greenberg HB, Robinson WS. The structure of hepatitis B Dane particle and nature of endogenous DNA polymerase reaction. J. Virol. 1977, 23:368-376
Marion PL, Knight SS, Salazar FH, Popper H, Robinson WS. Ground squirrel hepatitis virus infection. Hepatology 1983, 3:519-527
한국산 다람쥐에서의 Hepadna virus 감염

서울대학교 의과대학 간연구소*, **, 내과학과*** 및 중앙대학교 의과대학 내과학과***

유병철* · 김정룡**

한국에 서식하는 동물종에서 hepadna 바이러스의 유행을 억제하는 목적으로 한국산 다람쥐의 혈청에서 B형 간염 바이러스의 표지가 및 바이러스 염사를 검색하였다.

그 결과 47마리의 다람쥐종 6마리에서 HBsAg이, 7마리에서 anti-HBs가 양자면역측정법으로 검출되었다. HBsAg이 검출된 혈청을 전자현미경으로 관찰한 결과 21 nm직경의 구형입자들이 다수 발견되었으며 모든 직경이 41 nm이고 중앙에 핵이 있는 구형입자들이 발견되었다.

혈청에서 HBsAg과 구형입자들이 발견된 동물의 간조직을 병리학적으로 검사한 결과 간절

한국산 다람쥐에 hepadna 바이러스의 감염이 존재함을 알 수 있었다.