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Evidence of a Hepadna Virus Infection in Manchurian Chipmunks†

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= **Abstract** = In attempting to find a new host of hepadna 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 hepadna 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

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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 woochuck 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 occurence of persistent infection.

HBV and the three related viruses of lower animals are now called the hepadna viridiae (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, Ill.)

3. Detection of particles by electron microscope

To test for the presence of particles similar to HBV in chipmunks sera, sera were pelletted 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 parrafin, 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 ^{125}I-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 nec-

Table 1. Testing of sera of Chipmunks for HBV markers

	No. of positive sera	%
HBsAg	6	12.8
anti-HBc	0	0
anti-HBs	7	15.8
	Total 47	

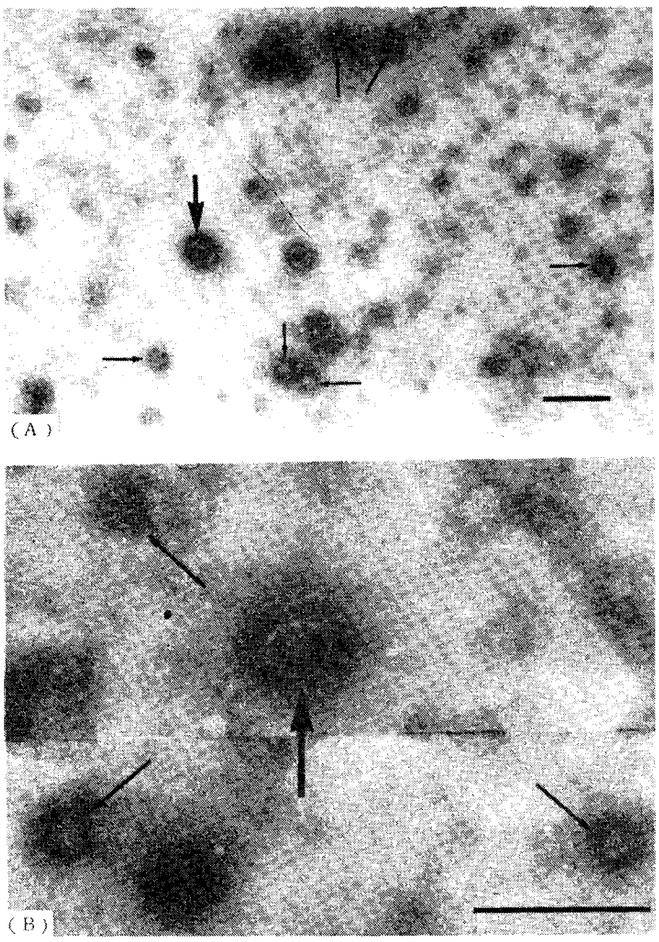

Fig. 1. Electron micrograph of particles in HBsAg-reactive sera of chipmunks. (A: animals no.41 B: animal no.33)
Large arrow; Dane particles-like spherical double shelled particles (41 nm)
Small arrow; Small spherical particles (21 nm)
Bar represents 100 nm

rosis, infiltration of inflammatory cells or fibrosis (Fig. 2).

DISCUSSION

We have found evidences of a hepadna virus infection in Manchurian chipmunks. Detection of weak HBsAg reactivities in sera of these animals

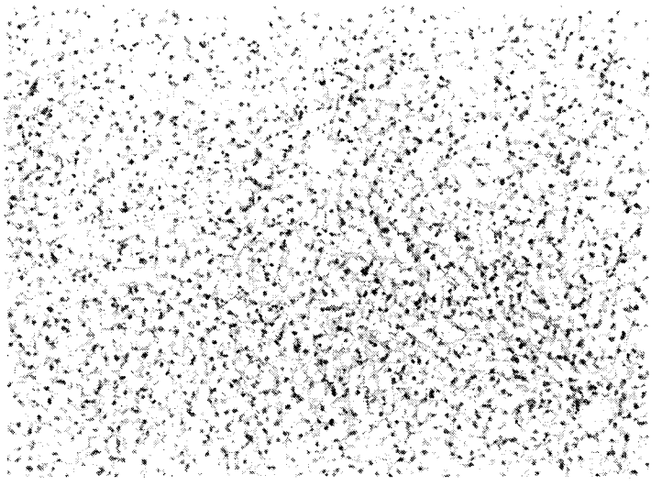

Fig. 2. Hematoxylin/Eosin-stained sections of liver from chipmunk, which contains HBsAg reactivity and virus-like particles in serum

strongly suggests a hepadna virus 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 small 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 hepadna virus infection, and other characteristics of the hepadna 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 2. Testing of sera of Chipmunks for HBV-like particles

Animal No.	HBsAg RIA S/N ratio	21 nm particles	41 nm particles
11	2.4	numerous	rare
14	2.3	numerous	rare
33	4.4	numerous	many
41	7.0	numerous	many
42	8.9	numerous	many
43	5.8	numerous	many

Sciuridae family like woodchucks and ground squirrels, the virus-like particles found in sera of chipmunks 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 chipmunks containing HBsAg and virus-like particles in sera revealed no evidence of liver disease, so these animals were considered to be healthy carriers. But longterm follow-up exam of chipmunks bearing virus-like particles would be required to clarify the existence of associated liver disease.

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= 국문초록 =

한국산 다람쥐에서의 Hepadna virus 감염

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한국에 서식하는 동물중에서 hepadna 바이러스의 숙주를 찾으려는 목적으로 한국산 다람쥐의 혈청에서 B형 간염 바이러스의 표지자 및 바이러스 입자를 검색하였다.

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

혈청에서 HBsAg과 구형입자들이 발견된 동물의 간조직을 병리학적으로 검사한 결과 간질환의 증거는 관찰되지 않았다.

혈청내에서 HBsAg과 교차반응하는 항원이 검출되고 B형 간염 바이러스의 virion 및 표면항원입자와 유사한 입자들이 관찰되어 한국산 다람쥐에 hepadna 바이러스의 감염이 존재함을 알 수 있었다.