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Detection of Hepatitis B Virus DNA in Pancreas, Kidney and Skin of Two Human Carriers of the Virus

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SUMMARY

Using the Southern blot technique, we have analysed the presence and state of hepatitis B virus (HBV) DNA in non-hepatic tissue from two human HBV carriers. HBV DNA sequences were detected in the pancreas, kidney and skin, demonstrating HBV infection of these organs. Moreover, the restriction DNA patterns were consistent with the integration of these viral sequences into high molecular weight DNA. These results demonstrate that HBV infection is not restricted to the liver.

Hepatitis B virus (HBV) infects only humans and chimpanzees and is considered to be strictly hepatotropic. In the liver of chronic HBV carriers with or without hepatocellular carcinoma (HCC), integrated HBV DNA sequences have been observed (Bréchet *et al.*, 1981*a*; Shafritz *et al.*, 1981; Chen *et al.*, 1982). The restriction enzyme patterns obtained from cellular DNA showed the presence of delineated HBV-specific bands. HBV DNA is therefore a new marker in the study of the relationship between the viral infection and chronic liver diseases. Recent studies have demonstrated the presence of the hepatitis B surface antigen (HBsAg) in pancreatic secretions during pancreatic stimulation (Hoefs *et al.*, 1980) and both the HBsAg and the hepatitis B core antigen (HBcAg) in the cytoplasm of pancreatic acinar cells (Shimoda *et al.*, 1981). It was therefore of interest to investigate the possibility of HBV infection in non-hepatic tissues. For this purpose we have used the Southern blot technique (Southern, 1975; Wahl *et al.*, 1979) with cloned viral DNA (Charnay *et al.*, 1979) as a probe to detect HBV sequences in the DNA of various organs.

Tissue samples were obtained at autopsy from two patients. Patient A was an 80 year old woman who died of cirrhosis with HCC. HBsAg, antibodies against the core antigen (anti-HBc) and the e antigen (anti-HBe) were present in her serum. The hepatitis e antigen (HBeAg) was not detected. Histological examination showed a normal pancreas and kidney without metastasis; of the liver only tumorous tissue was available. Patient B was a 67 year old man who died 5 months after the onset of a severe protracted acute hepatitis with massive renal failure due to glomerulonephritis. The acute hepatitis had occurred 2 months after blood transfusion during total pancreatectomy for a pancreatic adenocarcinoma. HBsAg, HBeAg and anti-HBc were present in the serum. A small pancreatic metastasis in the liver was carefully separated from the non-tumorous liver (as checked by histological examination). The kidney histology showed a membranous glomerulonephritis. HBsAg and HBcAg were detected in the tissues by an indirect immunoperoxidase technique using a murine monoclonal anti-HBs and human anti-HBc antibodies (Sternberger, 1979).

Cellular DNAs extracted from various organs of the two patients were digested with either *EcoRI*, which cleaved most HBV genomes once, or *HindIII*, which does not cut any of the HBV genomes so far analysed (Wain-Hobson *et al.*, 1982). For patient A (HBsAg-positive, HBeAg-negative in the serum) the *EcoRI* fragment hybridization patterns of the liver, the pancreas, the kidney and the skin (Fig. 1*a* to *d*) showed a unique band at the cloned HBV DNA position

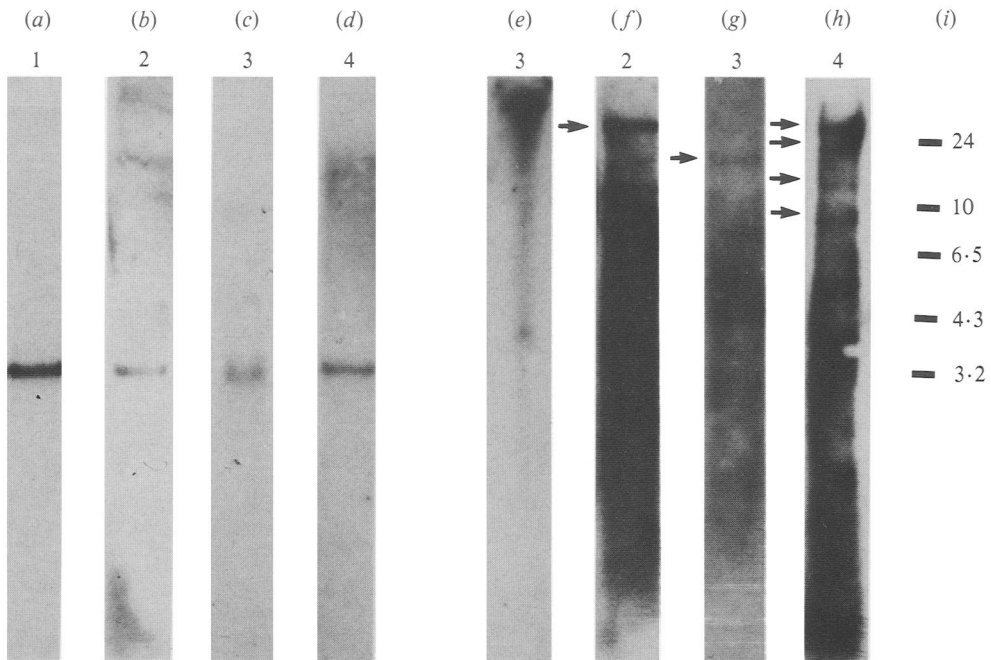


Fig. 1. Southern blot of tissue samples from patient A. DNAs of the different tissues were extracted by SDS/proteinase K digestion (Bréchet *et al.*, 1981*a*). Cellular DNAs were digested with *Eco*RI and *Hind*III (New England Biolabs). The DNA samples were electrophoresed on a 0.8% agarose gel in Tris-acetate buffer for 14 to 16 h at 1 V/cm. Serum DNA was electrophoresed similarly but without prior digestion. For serum analysis, HBV DNA was extracted from a pellet of centrifuged serum as previously described (Bréchet *et al.*, 1981*b*). DNA was then transferred to a nitrocellulose filter by a modification of the Southern technique described by Wahl *et al.* (1979). The filter was hybridized with 32 P-labelled, nick-translated cloned HBV DNA (Charnay *et al.*, 1979); the specific activity was $> 2 \times 10^8$ ct/min/ μ g. After extensive washing under stringent conditions ($0.1 \times$ SSC, 50°C), the filter was autoradiographed using Kodak XAR-5 film with a DuPont Lightning Plus screen for 1 to 3 days at -70°C . To avoid false positive results due to hybridization of the probe with bacterial DNA or bacterial DNA elements (transposons or plasmids) which often contaminate autopsy samples, we used an HBV probe purified twice by gel electrophoresis from the vector sequences (pBR322). In addition, each filter was dehybridized (Thomas, 1980) and rehybridized with a pure pBR322 probe. (a to d) 60 μ g *Eco*RI-digested cellular DNA extracted from liver (a), pancreas (b), kidney (c) or skin (d); (e) 40 μ g undigested kidney cellular DNA; (f to h) 60 μ g *Hind*III-digested cellular DNA extracted from pancreas (f), kidney (g) or skin (h); (i) reference sizes expressed in kilobases of linear DNA fragments obtained from *Hind*III digestion of λ plac5c185757. The small arrows indicate bands. The numbers 1, 2, 3, 4 correspond respectively to the liver, the pancreas, the kidney and the skin samples.

(3.2 kb). The *Hind*III patterns (Fig. 1*f* to *h*) revealed bands corresponding to large DNA fragments: at the 30 kb position for the pancreas, 20 kb for the kidney and from 9 to over 30 kb for the skin. For the liver, we could not get a clear result owing to the poor condition of the DNA. Hybridization of undigested cellular DNA took place in very high mol. wt. DNA. A representative result is shown in Fig. 1(*e*). HBV DNA sequences were not detected in the other organs analysed (Table 1). HBsAg was not detected in the tumorous liver or in the kidney but was present in small amounts in pancreatic acinar cells. HBcAg was never detected.

For patient B (HBsAg- and HBeAg-positive in the serum) the hybridization pattern of the undigested serum DNA (Fig. 2*h*) showed a very strong signal with one band at the 3.2 kb position and a short smear underneath, representing complete viral particles (Bréchet *et al.*, 1981*b*). In all the organs studied except the liver (i.e. kidney, skin, intestine, heart, brain and lung) a faint and short smear starting at the 3.2 kb position was detected as observed in the serum. Representative results are shown in Fig. 2(*i*, *j*). This result probably reflected blood

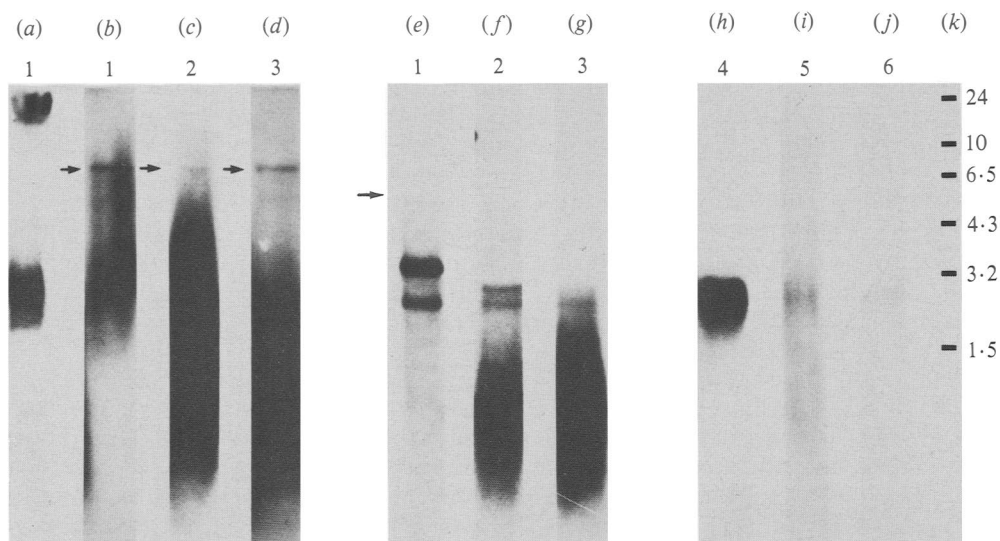


Fig. 2. Southern blot of tissue samples from patient B. (a) 40 μ g undigested kidney cellular DNA; (b to d) 60 μ g *Hind*III-digested cellular DNA extracted from kidney (b), non-tumorous liver (c) or liver pancreatic metastasis (d); (e to g) 60 μ g *Eco*RI-digested cellular DNA extracted from kidney (e), non-tumorous liver (f) or liver pancreatic metastasis (g); (h) 300 μ l serum; (i, j) 60 μ g undigested cellular DNA extracted from heart (i) or intestine (j); (k) reference sizes, as in Fig. 1. The small arrows indicate bands. The numbers 1, 2, 3, 4, 5, 6 correspond respectively to the kidney, the non-tumorous part of the liver, the liver pancreatic metastasis, the serum, the heart and the intestine samples.

Table 1. *HBV* DNA in tissues and serum

| | Patient A HBsAg ⁺ , HBeAg ⁻ , anti-HBc ⁺ | | Patient B HBsAg ⁺ , HBeAg ⁺ , anti-HBc ⁺ | |
|-----------|--|------------|--|------------|
| | Free | Integrated | Free | Integrated |
| Serum | — | — | + | — |
| Liver | — | + | + | + |
| Kidney | — | + | + | + |
| Pancreas | — | + | NA† | NA |
| Skin | — | + | + | — |
| Heart | — | — | + | — |
| Brain | — | — | + | — |
| Lung | — | — | + | — |
| Stomach | — | — | NT† | NT |
| Spleen | — | — | NT | NT |
| Intestine | NT | NT | + | — |

* Free and integrated HBV DNA was observed both in the non-tumorous liver and in the liver pancreatic metastasis.

† NT, Not tested; NA, not available.

contamination. In the liver an intense band at the 3.2 kb position with a long smear underneath and a few superimposed bands were observed in the *Hind*III (Fig. 2c) and *Eco*RI (Fig. 2f) DNA hybridization patterns. Such a pattern is considered to reflect viral multiplication (Bréchet *et al.*, 1981b). In addition, a unique band at the 8 kb position was observed after *Hind*III digestion in three samples: kidney, non-tumorous liver and liver pancreatic metastasis (Fig. 2b to d). The *Eco*RI DNA hybridization pattern of the kidney showed one faint band at 5.2 kb and two intense bands at 3.4 and 2.5 kb (Fig. 2e). The *Eco*RI DNA hybridization pattern of the non-tumorous part of the liver and the liver pancreatic metastasis showed, with the same intensity, a

3.2 kb band and a long smear underneath with superimposed bands (Fig. 2*f, g*). When uncut cellular liver and kidney DNAs were analysed, hybridization occurred in very high mol. wt. DNA. A representative result is shown in Fig. 2(*a*). HBsAg was detected in some liver cells but not in the kidney; HBeAg was never detected. The results obtained with patient B are summarized in Table 1. No hybridization was observed when DNA extracted from various organs obtained from a normal patient at autopsy were studied.

Altogether, these results demonstrate HBV infection of the kidney, the pancreas and the skin. The presence of HBV DNA in these tissues cannot be explained by blood contamination of the samples since the uncut and *Hind*III-digested DNA patterns are consistent with the integration of HBV DNA into high mol. wt. DNA. This is in accordance with the recent finding, in Pekin ducks, of pancreatic and kidney infection by the duck hepatitis B virus (DHBV), an HBV-like virus (Halpern *et al.*, 1983).

In addition, the restriction patterns observed suggest that HBV DNA may be integrated at particular sites in the cellular DNA. Indeed, after digestion by *Hind*III, which does not cut HBV DNA, distinct bands at a high mol. wt. DNA position were observed in the samples of liver and non-hepatic tissues both at an early (patient B) and late (patient A) stage of HBV infection. Furthermore, in the *Hind*III pattern from patient B a band was observed at the same position in the liver, the kidney and the liver pancreatic metastasis samples. The presence in two patients of a unique 3.2 kb band in most of the *Eco*RI patterns implies the existence of at least one complete viral genome whether or not rearrangements may be involved. A restriction DNA pattern of this kind is often observed in HBsAg-positive, HBeAg-negative patients with chronic hepatitis (Br  chot *et al.*, 1981*a*). The reason for the absence in the *Eco*RI DNA pattern of other bands arising from flanking sequences remains unclear and will only be resolved by molecular cloning procedures.

For patient B intense viral multiplication was present only in the liver, both in the pancreatic metastasis tissues and in the non-tumorous tissues. Although one cannot exclude contamination by the surrounding hepatocytes, this suggests that viral multiplication could occur in pancreatic cells. This would be in accordance with the demonstration of HBeAg in the pancreas of some HBV carriers (Shimoda *et al.*, 1981) and of DHBV multiplication in the pancreas of viraemic ducks (Halpern *et al.*, 1983). By contrast, we did not detect either viral multiplication or HBsAg and HBeAg in the kidney despite the presence of HBV DNA sequences.

Since HBV DNA was detected in only a few organs of these patients, horizontal rather than vertical viral transmission is likely. This raises a problem concerning the mechanism of HBV penetration of the cells: HBV-specific receptors have only been localized in the liver (Lutwick *et al.*, 1982). Finally, pancreatic, renal and skin lesions have been described in HBV chronic carriers (Nowoslawski, 1981). Thus, the presence of extra-hepatic HBV DNA sequences may be involved in the pathogenesis of these lesions.

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