Comparative Expression of Hepatitis B Virus Antigens in Several Cell Model Systems

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Summary

In this paper the kinetics of hepatitis B virus (HBV) gene expression were investigated in natural and experimentally transfected cell systems. These systems included four human hepatocellular carcinoma cell lines containing HBV DNA (TONG/PHC, HEp 3B2, PLC/PRF/5 and HA22T/VGH) as well as a mouse and a rat cell line both experimentally transfected with HBV DNA. Comparative results on the kinetics of hepatitis B surface antigen in these cell systems suggested that the S gene in the integrated state is expressed at different levels. No human cell line derived from HBV-associated hepatocellular carcinoma produced hepatitis B e antigen (HBeAg) when medium was concentrated by ultrafiltration. In distinct contrast, the two experimentally transfected cell systems produced e antigen at different levels. When all HBV-containing cell lines were grown as tumours in nude mice, no HBeAg was detected in the serum of these mice inoculated with human hepatocellular carcinoma cell lines, in the tumour homogenates, or in the tumour-derived lines, whereas e antigen was expressed both in vivo and in vitro in the experimentally transfected cell lines. These observations indicate that C gene expression is restricted to transfected cell cultures and this suggests a distinct difference in the mechanisms of HBV gene expression between the two types of in vitro model systems.

Introduction

Hepatitis B virus (HBV) causes serious liver disease in man ranging from acute to persistent infection and may lead to the development of primary liver cancer (MacSween, 1980). It is estimated that approximately 4% of the world population are chronically infected with hepatitis B virus (Szmuness, 1978). In various parts of the world, such as Africa and Asia, the HBV carrier rate may approach 10 to 15% of the population (Kew, 1981). HBV DNA integrates into liver cell chromosomal DNA during the course of chronic HBV infection, prior to clinical detection of hepatocellular carcinoma (HCC) (Brechot et al., 1981; Shafritz & Kew, 1981). However, it is not known how integration of HBV DNA may lead to the formation of primary liver cancer.

HBV consists of an outer protein coating, the hepatitis B surface antigen (HBsAg), and a nucleocapsid comprising the hepatitis B core antigen (HBcAg). The viral core contains the viral DNA as well as a DNA polymerase, a protein kinase activity and a DNA-linked protein. (For review, see Tiollais et al., 1985.) Another protein (Magnius & Espmark, 1972), termed hepatitis B e antigen (HBeAg), is found in soluble form in sera of patients when virus is actively produced, either during acute or chronic infection (Nordenfeldt & Kjellen, 1975). Treatment of core particles of HBV with 2-mercaptoethanol and heat, or mild protease digestion, results in the conversion of core antigen into the closely related e antigen (Takahashi et al., 1979; MacKay et al., 1981). Thus it is believed that e Ag is a derivative of c Ag. The existence of dimeric or multimeric forms of HBeAg has been shown (Serrano & Hirschman, 1984; Takahashi et al., 1981), and the proclivity of HBeAg to form multimers makes it likely that this protein is a structural building block of the viral core.
All attempts to propagate HBV in vitro have so far failed and the lack of such a system has greatly hampered a detailed study of viral gene expression during virus replication, and thus knowledge about the production of HBV antigens is rather limited. However, systems for incomplete virus replication exist, and in general three different approaches have been used to study HBV expression: (i) transcription in vitro of cloned HBV DNA (Chakraborty et al., 1981), (ii) expression of HBV genomes integrated into chromosomal DNA of human, mouse and rat cells (Aden et al., 1979; Alexander et al., 1978; Dubois et al., 1980; Edman et al., 1980; Gough & Murray, 1982; Serrano & Hirschman, 1984), and (iii) expression of HBsAg using viral vectors (Moriarty et al., 1981; Liu et al., 1982; Wang et al., 1983). We have used the second method to study HBV gene expression.

In this report we compare the kinetics of HBV gene expression in natural and experimentally transfected cell systems with different degrees of viral antigen production. These systems include four human hepatocellular carcinoma cell lines containing HBV DNA, and a mouse and a rat cell line both experimentally transfected with HBV DNA. HBsAg and HBeAg expression was investigated in all the cell lines by comparing the production of these antigens during the growth cycle of the cells.

Since it has been shown that expression of HBV DNA integrated into host cell chromosomes is inducible by changes in the environment (Marquard et al., 1984), we investigated HBV gene expression in all the cell systems when grown as tumours in nude mice as well as in cell lines subsequently derived from these tumours, thus comparing HBV gene expression in vitro and in vivo.

The results provide a comparison of HBV gene expression in human hepatoma cell lines derived from naturally occurring tumours, as well as a comparison between the ‘natural’ situation and experimentally transfected systems.

METHODS

Cell lines. See Table 1.

Culture conditions. All cell lines were grown as monolayer cultures in 75 cm² tissue culture flasks at 37 °C in Eagle's MEM (Gibco) supplemented with 4-67 g/l HEPES and 10% inactivated foetal calf serum. TONG/PHC cells were cultivated in the same medium, but supplemented with 1% foetal calf serum. Antibiotics were added as described previously (Alexander et al., 1976). Twenty ml of medium was used per 75 cm² flask. All cell lines were subcultured by suspension in 10 ml trypsin-EDTA solution [0.05% EDTA, 0.05% glucose, 0.125% trypsin in calcium- and magnesium-free phosphate-buffered saline (PBS)]. Mouse L cells were subcultured in trypsin solution (0.25% trypsin in PBS) only. Stock cultures were serially passaged at a 1:8 split ratio. Trypsinized cells, diluted 1:20 in Coulter Isoton II were counted in a Coulter Counter Model ZM with a 70 μm aperture tube.

HBV antigen determinations. HBsAg in cell growth medium, in the sera from nude mice and in tumour homogenates were measured by AUSRIA (Abbott). HBeAg was determined by a HBeAg radioimmunoassay (RIA) test (Abbott) or SB-EB monoclonal e antigen kit (SORIN). RIA counts were obtained from a ANSR Gamma Counter (Abbott Laboratories). Positive/negative sample ratios (P/N) >2.1 were taken as positive.

Status of HBV gene expression in the cell lines in vitro. Fifteen ml growth medium was harvested from all cell lines 7 days after seeding and concentrated using a Millipore immersible CX ultrafiltration system (10000 mol. wt. cut-off) and assayed for HBsAg and HBeAg. For comparative HBsAg production on a per cell basis, the amounts of HBsAg were calculated with reference to known standards.

HBsAg and HBeAg production in vitro. TONG/PHC, Hep 3B, PLC/PRF/5, L HBV and BM3 cultures were each seeded at 5 x 10⁶ cells per flask in fourteen 25 cm² flasks (duplicate cultures to monitor HBsAg production over 7 days). Twenty-four h later all flasks were changed with fresh growth medium to eliminate any possible trypsin effects on the cells. This was regarded as day 0. Each day thereafter for 7 days, medium was harvested from two flasks for assaying HBsAg. Cells were removed from the same flasks and counted. All remaining flasks were re-fed with fresh growth medium. Values presented represent the average of duplicate flasks sampled.

L HBV and BM3 cells were seeded at approximately 50% confluence in 75 cm² tissue culture flasks. Growth medium was changed after 24 h with 15 ml fresh medium. Three days later 15 ml of growth medium was harvested from each flask of each cell line and cells were counted. Fresh medium was added to the remaining flasks. This procedure was repeated on day 5 and day 7. All harvested samples were concentrated two-, five- and tenfold by ultrafiltration, using a Millipore immersible CX ultrafiltration system, 10000 mol. wt., and assayed for HBeAg. The 10000 mol. wt. cut-off filter units were chosen to ensure the retention of the low mol. wt. e antigen, as well as multimeric forms, which may be present in the culture fluids.
Patient A
Age 59 M Italian
Sex NS* 8 M American
Origin NS 24 M Mozambique
+ NS
56 M Chinese
HS Ag HBe Ag Designation HBV DNA HBs Ag HBe Ag
TONG/PHC + + - + + - (Lin et al., 1984)
HEp 3B2 + + -
PLC/PRF/5 + + -
HA22T/VGH + + - + + -
Mouse L cells (L HBV) + +
Buffalo rat liver cells (BM3) + +

* NS, Not screened.
† C. Chang, personal communication.

Table 1. Cell lines used in this study

RESULTS
Status of HBV gene expression in the cell lines in vitro

Since HBeAg was recently detected at low levels in concentrated growth medium from L HBV cells (Aspinall & Alexander, 1985), the growth medium from all the cell lines in this study was concentrated and assayed for HBeAg. Table 2 shows the HBsAg and HBeAg activity in the cell lines. All cell lines investigated except the HA22T/VGH line were positive for HBsAg. None of the human hepatoma cell lines expressed detectable HBeAg, whereas in the experimentally transfected cell lines (L HBV and BM3), HBeAg was measured. On a per cell basis the PLC/PRF/5 and BM3 cells produced the highest amounts of HBsAg.

Nude mouse studies. Athymic nude mice, MF1 nu nu/WITS (obtained from a breeding colony at the University of the Witwatersrand Animal Research Centre) were maintained under isolation conditions in a laminar flow hood. Cages, bedding and water were autoclaved. Water was supplemented with 0.8 g/l terramycin and 1.35 g/l potassium sorbate. Mice cubes were sterilized by radiation. For all experiments 4- to 8-week-old mice were used.

Cells were grown in 75 or 150 cm² tissue culture flasks to confluence, removed from the surfaces with 10 to 15 ml trypsin-EDTA solution, counted and pelleted. Cells were resuspended in 0.5 ml serum-free MEM in a 1 ml tuberculin syringe and injected subcutaneously into the back of a nude mouse. Inoculated mice were observed daily for tumour growth.

At termination of the experiment, the mice were weighed, anaesthetized with Sagatal intraperitoneally (0.08 mg/g mouse) and bled by cardiac puncture. An average of 0.5 to 1 ml of whole blood was usually obtained. Two-hundred to 400 l.d of serum per mouse was recovered from blood and stored frozen at -70 °C until used for detection of HBV markers.

Tumours were excised under sterile conditions and weighed. One portion of each tumour was used for re-establishing cell lines and another portion for tumour homogenization. Using aseptic technique, a section of each tumour was cut with scissors into 0.1 to 1 mm³ pieces and softened by trituration through a needle with growth medium and inoculated into tissue culture flasks. For each tumour, six flasks (25 cm²) were used containing approximately 2 ml each of suspended tumour tissue in growth medium. Medium was harvested from each tumour-derived cell line when confluent after the first passage and assayed for HBsAg and HBeAg.

A section of each tumour as well as a piece of liver from a control uninoculated nude mouse and human liver were rinsed repeatedly with PBS until no further blood was removable. Each was homogenized gently in a glass tissue homogenizer in approximately 2 ml hypotonic buffer per g tissue (10 mM-Tris–HCl pH 7.5, 10 mM-KCl, 1.5 mM-magnesium acetate). Homogenized tumour cells were centrifuged in an Eppendorf centrifuge (5413) for 10 min. One-hundred and fifty μl supernatant from each homogenate was incubated separately with PBS and sera with different HBV markers, i.e. anti-e-positive serum, anti-core-positive serum and HBV marker-negative serum, before adding the anti-HBeAg coated beads for the HBeAg RIA test.
Fig. 1. Kinetics of HBsAg production (△) compared to cell number (●). (a) TONG/PHC, (b) HEp 3B2, (c) PLC/PRF/5, (d) L HBV, (e) BM3. The horizontal broken lines in (a), (b) and (d) represent the cut-off value.

Table 2. Radioimmunoassay for hepatitis B surface and e antigens in cell culture fluids

<table>
<thead>
<tr>
<th>Material tested*</th>
<th>HBsAg (ng ml/10⁶ cells/24 h)</th>
<th>HBeAg†</th>
</tr>
</thead>
<tbody>
<tr>
<td>TONG/PHC</td>
<td>6.5</td>
<td>0.92</td>
</tr>
<tr>
<td>HEp 3B2</td>
<td>5.0</td>
<td>0.78</td>
</tr>
<tr>
<td>PLC/PRF/5</td>
<td>93.6</td>
<td>1.15</td>
</tr>
<tr>
<td>HA22T/VGH</td>
<td>0</td>
<td>1.09</td>
</tr>
<tr>
<td>L HBV</td>
<td>17.0</td>
<td>1.50</td>
</tr>
<tr>
<td>BM3</td>
<td>100.0</td>
<td>8.80</td>
</tr>
<tr>
<td>Growth medium control</td>
<td>0</td>
<td>0.97</td>
</tr>
</tbody>
</table>

* This comparison was done on 1-day-old supernatant medium from the cells, 7 days after seeding. The amounts of HBsAg were calculated with reference to known standards and computed to represent HBsAg produced by 10⁶ cells.

† HBeAg was measured at two concentrations by the Abbott HBeAg/Ab test kit: P/N > 2.1 taken as positive.

Kinetics of HBsAg production in vitro

Fig. 1 indicates that there was a difference in the kinetics of HBsAg gene expression in these cell lines. The PLC/PRF/5, L HBV and BM3 lines produced measurable levels of HBsAg throughout the growth cycle, whereas the TONG/PHC and HEp 3B2 cells only produced HBsAg when cells approached the stationary phase. In PLC/PRF/5, L HBV and BM3 cells HBsAg production increased concomitantly with the number of cells whereas this was not demonstrated for the TONG/PHC and HEp 3B2 cell lines.

Kinetics of HBeAg production in vitro

Fig. 2 shows the production of HBeAg in the L HBV and BM3 cells during various phases of the growth cycles. The BM3 cells transfected with closed circular monomeric HBV DNA
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Fig. 2. HBeAg production from L HBV (▲) and BM3 (○) cells. Cells were seeded as described in Methods, and HBeAg was assayed using the Abbott HBeAg diagnostic kit. P/N of test, 9.85. The broken horizontal line represents the cut-off value. In each group of three curves, values are plotted for cells in the stationary, late exponential and logarithmic phases from top to bottom.

Table 3. HBV gene expression in serum of nude mice carrying subcutaneous tumours

<table>
<thead>
<tr>
<th>Number of injected cells</th>
<th>Tumour appearance (days) †</th>
<th>Tumour dissection (days) †</th>
<th>Tumour weight as % total body weight</th>
<th>Serum of mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HBSAg P/N ‡</td>
</tr>
<tr>
<td>1.2 \times 10^7 TONG/PHC</td>
<td>26</td>
<td>34</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>8.7 \times 10^6 TONG/PHC</td>
<td>34</td>
<td>108</td>
<td>63.7</td>
<td>64.7</td>
</tr>
<tr>
<td>1.8 \times 10^7 HEp 3B2</td>
<td>22</td>
<td>28</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>1.1 \times 10^7 HEp 3B2</td>
<td>42</td>
<td>53</td>
<td>7.9</td>
<td>161.9</td>
</tr>
<tr>
<td>9.9 \times 10^6 PLC/PRF/5</td>
<td>24</td>
<td>31</td>
<td>7.5</td>
<td>147.2</td>
</tr>
<tr>
<td>1.2 \times 10^7 PLC/PRF/5</td>
<td>14</td>
<td>28</td>
<td>8.2</td>
<td>109.2</td>
</tr>
<tr>
<td>1.4 \times 10^7 HA22T/VGH</td>
<td>113</td>
<td>207</td>
<td>2.7</td>
<td>1.2</td>
</tr>
<tr>
<td>1.0 \times 10^8 L HBV</td>
<td>26</td>
<td>32</td>
<td>3.0</td>
<td>1.4</td>
</tr>
<tr>
<td>1.3 \times 10^7 L HBV</td>
<td>25</td>
<td>36</td>
<td>12.4</td>
<td>1.5</td>
</tr>
<tr>
<td>8.7 \times 10^6 BM3</td>
<td>133</td>
<td>161</td>
<td>5.6</td>
<td>121.4</td>
</tr>
</tbody>
</table>

* HBeAg was measured with the SB-EB monoclonal RIA kit (SORIN).
† Number of days after cells had been injected.
‡ P/N > 2.1 is positive.

produced approximately four times more HBeAg than the mouse L cells with the dimeric form of HBV DNA. The results indicate that the production of HBeAg was low in actively dividing cells and no e antigen was detected during the logarithmic phase of growth in the L cells. In both cell lines the production of HBeAg increased in the late exponential and stationary phases.

Gene expression of HBV-containing cell lines in nude mice (in vivo) and in vitro

Table 3 shows that the serum of nude mice inoculated with TONG/PHC, HEp 3B2, PLC/PRF/5 and BM3 cells was positive for HBsAg. Generally, HBsAg could not be detected in the sera of nude mice when the tumour sizes were small. No HBeAg was detected in the serum of mice inoculated with human HCC cell lines.
Table 4. *HBeAg (RIA) detection in tumour and control liver homogenates*

<table>
<thead>
<tr>
<th>Homogenate</th>
<th>Homogenate</th>
<th>Homogenate</th>
<th>Homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ PBS</td>
<td>+ anti-E(+) serum</td>
<td>+ anti-core (+) serum</td>
<td>+ HBV (-) serum</td>
</tr>
<tr>
<td>Natural models</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TONG/PHC tumour</td>
<td>2.4</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td>HEp 3B2 tumour</td>
<td>1.8</td>
<td>1.8</td>
<td>1.2</td>
</tr>
<tr>
<td>PLC/PRF/5 tumour</td>
<td>2.3</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>HA22T/VGH tumour</td>
<td>2.9</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Experimental models</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L HBV tumour</td>
<td>2.5</td>
<td>1.3</td>
<td>2.2</td>
</tr>
<tr>
<td>BM3 tumour</td>
<td>56.0</td>
<td>1.5</td>
<td>40.6</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control mouse liver</td>
<td>3.2</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Control human liver</td>
<td>2.3</td>
<td>2.0</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*P/N values in test runs using the Abbott HBeAb diagnostic kit were >11.0. P/N > 2.1 is positive.

Table 5. *Radioimmunoassay (P/N)* for hepatitis B surface and e antigen in cell culture fluids from nude mouse tumour-derived cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>HBsAg 1x</th>
<th>10x</th>
<th>HBeAg† 1x</th>
<th>10x</th>
</tr>
</thead>
<tbody>
<tr>
<td>TONG/PHCm</td>
<td>1.48</td>
<td>57.9</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>HEp 3B2m</td>
<td>1.49</td>
<td>37.0</td>
<td>1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>PLC/PRF/5m</td>
<td>56.20</td>
<td>129.7</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>HA22T/VGHm</td>
<td>1.24</td>
<td>0.79</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>L HBVm</td>
<td>51.90</td>
<td>87.3</td>
<td>3.3</td>
<td>9.6</td>
</tr>
<tr>
<td>BM3m</td>
<td>88.90</td>
<td>148.6</td>
<td>16.0</td>
<td>90.6</td>
</tr>
</tbody>
</table>

*P/N = c.p.m. experimental/c.p.m. negative control (P/N > 2.1, positive).
†HBeAg was measured using the SB-EB monoclonal RIA kit (SORIN).

When tumour homogenates were assayed for HBeAg (Table 4), the reaction to e antigen shown by human tumour homogenates or control mouse liver or control human liver gave a non-specific reaction for e antigen as viral antibody-negative serum also reacted to produce a negative result. Specific HBeAg was detected in the L HBV (low levels), and BM3 tumour homogenates as HBeAg was specifically removed by the anti-e positive serum.

Table 5 shows HBV gene expression in nude mouse tumour-derived cell lines. No HBeAg was detected in any human tumour-derived cell line. This correlates with HBV gene expression of the original parental lines (see Table 2).

**DISCUSSION**

In this study two approaches were used to investigate HBV gene expression: human hepatoma cell lines (natural models) and a mouse and a rat cell line transfected with HBV DNA (experimental models).

Mouse L cells transfected with HBV DNA were originally reported to be HBcAg- and HBeAg-negative (Dubois et al., 1980), but we have recently shown low-level expression of e antigen by these cells (Aspinall & Alexander, 1985). When the growth medium was concentrated by ultrafiltration none of the cell lines derived from HBV-associated hepatocellular carcinoma in this study produced HBeAg, including the TONG/PHC cells which were derived from an e antigen-positive patient, whereas the experimental models (L HBV and BM3) do so (Table 2).

The kinetics of HBsAg production was monitored in HBsAg-positive cell lines, and the results indicated that there were differences in the expression of the HBs gene in these cell systems (Fig. 1). Generally, in all cell lines more HBsAg was produced during the stationary phase, which makes it likely that HBsAg production was inhibited during DNA replication. On a per cell basis the PLC/PRF/5 and the BM3 cell lines were the highest producers of HBsAg, followed by...
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L HBV cells. The HEp 3B2 and TONG/PHC cells were low producers of HBsAg. This suggests that the S gene in the integrated state was expressed at different levels.

Differences in the kinetics and the amounts of HBsAg produced by chromosomally integrated HBV DNA by the various cell systems may be due to the following circumstances. (i) The number of integration sites of HBV DNA into cellular DNA (HBV copy number) may correlate with the amounts of HBsAg produced (Edman et al., 1980; Twist et al., 1981). (ii) Different 3' sequences of the S gene may account for different levels in HBsAg expression (Freytag von Loringhoven et al., 1985). (iii) Different integration sites in these cell lines. (iv) Disruption of the coding region of the HBsAg gene may affect HBsAg production (Shaul et al., 1984). (v) Methylation of HBsAg regions of integrated HBV DNA sequences may account for differential expression (Miller & Robinson, 1983). (vi) Levels of HBsAg gene expression of integrated sequences may be dependent on the presence of an enhancer (Shaul et al., 1985). All these speculations can be tested in the laboratory and should yield more insight with regard to HBsAg gene expression in persistently infected carriers.

HBsAg and HBeAg were determined in the sera of mice bearing tumours, in tumour homogenates and in cell lines derived from these xenografts, to investigate whether all HBV-containing cell lines show the same pattern of gene expression when grown as tumours in nude mice and in tissue culture. Passage of the human cell lines in nude mice and subsequent reculturing of the tumour tissue did not lead to any e antigen expression, whereas e antigen was detected in vivo and in vitro in both experimental cell lines (Tables 3, 4 and 5). We conclude that the functional state of the HBV genome does not depend on the environment, as has been suggested by Marquardt et al. (1984), since HBV gene expression in the tumour homogenates and tumour derived cell lines did not differ from that measured in the parental cell lines.

In contrast to the human HCC cell lines, the experimentally transfected cell systems produced e antigen. Although Yoakum et al. (1983) reported HBcAg gene expression in the PLC/PRF/5 cells, we have been unable to repeat these findings, using normal in vitro conditions. No human cell line derived from HBV-associated hepatocellular carcinoma under normal conditions has yet been shown consistently to produce HBcAg or HBeAg, whereas an experimentally transfected human line does so (Korba et al., 1985). Thus it seems that constitutive C gene expression is restricted to transfected cell cultures.

The overall pattern emerging from these experiments is that cell lines derived from naturally occurring HCC express only HBsAg providing the S gene is present and that the ability to express the S gene is conserved, while C gene expression resulting in core antigen or e antigen production is not. Recently it has been shown that the pre-core region of the C gene targets the core antigen to cellular membranes and causes the secretion of e antigen (Ou et al., 1986). It is possible that integration of HBV in natural infections may occur within an HBV DNA locus that prevents C gene expression. The S gene can be transcribed from integrated sequences, but freely replicating viral genomes may be necessary for C gene expression. Recently Matsui et al. (1986) have grown human hepatocellular carcinoma, taken from biopsy material, as tumours in nude mice and demonstrated both HBsAg and HBeAg as well as core-like particles. The tumour cells contain both integrated and extrachromosomal forms of HBV DNA.

Thus at present there are three experimental situations. First, HBV–hepatocyte interactions leading to hepatocellular carcinoma from cell line studies indicate that HBV DNA is integrated and only HBsAg is constitutively produced. Second, transfected HBV DNA in both L cells and BM3 cells is integrated, but both HBsAg and HBeAg are produced. Third, hepatocellular carcinoma samples taken from a biopsy specimen produce transplantable tumours in nude mice which contain both integrated and extrachromosomal HBV DNA. The tumour cells are HBsAg- and HBeAg-positive and contain core-like structures.

It is conceivable that genetic rearrangements within the HBV genome might have taken place during the establishment of human hepatoma cell lines in vitro and may therefore represent a selected cell population. However, PLC/PRF/5, HEp 3B2 and TONG/PHC were each established by different methods as cell lines in vitro, and none produces e antigen. The establishment of a cell line from xenograft human HCC carried by nude mice (PLC/342), may demonstrate whether or not HCC in vitro is able to express both HBsAg and HBeAg.
We wish to thank Professor M. Tong for supplying the TONG/PHC cells, Professor B. Knowles for the HeP 3B2 cells, Professor C. Chang for the HA22T/VGH cells, Professor S. Hirschman for the BM3 cells, Professor P. Tiollais for HBV DNA-transfected mouse L cells, and Dr G. Dusheiko for the HBsAg standards. This work was supported by a grant from the South African Medical Research Council.

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