Woodchuck hepatitis virus surface antigen produced in vitro fails to bind polymerized woodchuck serum albumin

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Using a plasmid (pSWS) similar to one that has been successfully used for large-scale production of hepatitis B virus (HBV) envelope protein particles (pSVS) but containing the corresponding woodchuck hepatitis virus (WHV) envelope gene sequences, we have stably transformed the rodent dihydrofolate reductase-deficient cell line CHO dhfr⁻. Although production of WHV envelope particles in CHO/pSWS cell lines was low, it was sufficient to test whether these particles could bind to polymerized serum albumin. Whereas binding of HBV particles produced in CHO/pSVS cells to polymerized human serum albumin could readily be detected, we found no evidence that the WHV envelope protein particles produced in vitro bind to either human or woodchuck polymerized serum albumin.

Hepatitis B virus (HBV), an enveloped DNA virus of the Hepadnaviridae family, is responsible for polymorphic acute and chronic liver diseases as well as hepatocellular carcinoma (Robinson, 1990). Woodchuck hepatitis virus (WHV) is the hepadnavirus most closely related to HBV in terms of structure and pathological consequences of viral infection, and therefore constitutes an interesting model for HBV biology (Kay et al., 1988).

The narrow host range and the high hepatotropism of these viruses is one of their major characteristics. However, the molecular determination of these attributes is not as yet fully understood. It may be that cellular receptors that allow penetration of the virus are present only on target cells of permissive hosts. A model accounting for penetration restricted to hepatocytes has been proposed for HBV (Thung & Gerber, 1984). It is based on the in vitro observation of an interaction between HBV virions or envelope particles (HBsAg) and glutaraldehyde-polymerized human serum albumin (HpSA) (Machida et al., 1984). It has been suggested that this interaction could play a role in the hepatotropism of HBV because of the exclusive presence of polyalbumin receptors at the surface of hepatocytes. However, the role in vivo of such an interaction still awaits to be established.

A previous study (Pohl et al., 1986) using woodchuck hepatitis virus surface antigen (WHsAg) particle-enriched fractions from infected animal sera indicated that, unlike HBV, WHV particles did not bind to woodchuck polymerized serum albumin (WpSA). However, viral particles derived in vivo may be associated with serum proteins, including serum albumin itself or anti-WHsAg antibodies, which could mask WpSA binding sites, as has been observed with serum-derived HBV particles (Heerman et al., 1987; B. Shamoon, unpublished). This association of serum proteins with viral particles creates potential problems in any study involving viral particle interactions and the availability of particles derived in vitro should greatly assist.

We report here the construction of rodent recombinant cell lines that are stably transformed with WHV envelope gene sequences. These cell lines were used to produce in vitro WHV envelope particles which were studied for their interaction with WpSA.

The expression plasmid pSWS for the synthesis of WHV envelope proteins was derived from plasmid pSVS (Michel et al., 1984, kindly provided by Professor P. Tiollais). This plasmid was chosen because it has been used successfully for the large-scale production of HBsAg particles in CHO cells. It contains HBV sequences cloned downstream from a simian virus 40 (SV40) early promoter/enhancer element, as well as a cDNA sequence encoding a murine dihydrofolate reductase (dhfr) gene downstream from a mouse mammary tumour virus long terminal repeat promoter.

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By site-directed mutagenesis (Zoller & Smith, 1984) an EcoRV site was introduced into pSVS at the junction between the SV40 early promoter/enhancer element and the 5' end of the HBV sequence. pSVS contains an additional EcoRV site located immediately downstream from the 3' end of the HBV sequence, permitting complete excision of the HBV sequence.

An EcoRV site was also introduced into the WHV genome 14 nucleotides upstream from the ATG of preS1. Digestion of this altered genome with EcoRV and HindIII released a 2522 nucleotide fragment (from positions 2977 to 2190; numbering according to Galibert et al., 1982) containing WHV sequences equivalent to the HBV sequences in pSVS, i.e. the entire preS/S region, the X gene and part of the C gene which includes the polyadenylation site. After filling of the HindIII 5' end overhang, the fragment was cloned into the pSVS vector from which the HBV sequence had been excised, giving rise to pSWS. In this plasmid the large (preS1) surface antigen gene is under the transcriptional control of the SV40 early promoter/enhancer element and messengers for middle (preS2) and major (S) surface antigens should be produced from their natural (WHV) promoters.

Both pSVS and pSWS were transfected into the rodent cell line CHO dhfr− (kindly provided by Dr L. A. Chasin) that was propagated as described (Urlaub & Chasin, 1980). Transfection was performed by the polybrene method (Chaney et al., 1986). Cells (10⁶) were incubated with 2 μg plasmid DNA and 30 μg polybrene for 6 h; 48 h after transfection, the cells were incubated in selective MEM alpha medium. Both pSVS and pSWS transfectants appeared with the same efficiency (about 25 clones per μg DNA per 10⁶ CHO cells) and after the same selection period (about 15 days).

CHO dhfr+ clones were tested for the production of HBsAg or WHsAg in both the intracellular and extracellular compartments. HBsAg was detected using a commercial solid-phase radioimmunoassay (Austria II; Abbott). The same test was used for the detection of WHsAg given the cross-reactivity of anti-HBsAg antibodies with WHsAg (Millman et al., 1982). The quantity of WHsAg detected in CHO/pSWS cell lysates was however 400-fold lower than the HBsAg found for CHO/pSVS clones whereas the amounts of WHsAg found in the culture medium of CHO/pSWS clones are only fourfold lower than the amounts of HBsAg secreted by CHO/pSVS clones (Table 1). This suggests that in the pSVS cell lines, which are producing large quantities of HBsAg, secretion is a rate-limiting step.

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HBV and WHV envelope proteins synthesized by the recombinant clones were analyzed in typical empty particles of HBsAg and WHsAg, as shown by electron microscopy (Fig. 1). These particles were mostly 22 nm in diameter with few filaments of identical diameter and variable length.

Table 1. Expression of HBsAg and WHsAg particles by CHO transfectant clones

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Supernatant</th>
<th>Cell lysate</th>
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<tr>
<td>pSVS</td>
<td>248 ± 0.39 (n = 9)</td>
<td>4.6 ± 0.169 (n = 9)</td>
</tr>
<tr>
<td>pSWS</td>
<td>2.48 ± 0.029 (n = 9)</td>
<td>4.6 ± 0.169 (n = 9)</td>
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* Values represent signal/background ratios of c.p.m, as measured in the Austria II test with mean value ± S.E.M. given. n, Number of clones.

† Range.

Fig. 1. Electron micrographs of 10-fold-concentrated medium from a CHO/pSWS (a) and a CHO/pSVS (b) transfectant cell line. Specimens were stained with phosphotungstic acid. The bars represent 100 nm. For WHsAg particles a field containing both particles and filaments was chosen for illustration.
The glycosylation pattern of the recombinant WHsAg particles is thus different from that of natural particles which contain only the gp36 form of the preS2 envelope particles by amplifying integrated WHV sequences using increased resistance to methotrexate (Stark, 1984). This approach worked well with CHO/pSVS clones, but it failed with the CHO/pSWS clones (not shown). There is abundant transcription of WHV envelope protein mRNA sequences in these clones, this level being comparable with that of CHO/pSVS clones (not shown), and the two expected transcripts are present (one of 2.8 kb initiated at the SV40 early promoter/enhancer, and one of 2.1 kb initiated down-stream to the preS2 ATG). Transcriptional regulation therefore does not seem to account for the low levels of WHsAg produced. Efficient translation of WHsAg transcripts may require a cellular factor which is in limiting supply in CHO cells.

It is known that HBsAg can interact via its preS2 region with HpSA, a process which may be involved in allowing HBV to penetrate its target cells (Machida et al., 1984). Previous studies using WHsAg particles purified from sera of infected animals failed to detect a similar interaction between WHsAg and WpSA (Pohl et al., 1986). However, Heermann et al. (1987) have reported that HBV preS2 determinants can be masked in human serum. We have also observed that HBV particles purified from the serum of infected patients bind less efficiently (about 10-fold) to HpSA than particles produced in vitro (not shown). We therefore decided to test the interaction using WHsAg particles produced in vitro in the presence of only 1% fetal calf serum. WHsAg particle-enriched fractions (tested by Ausria II) were obtained by ultracentrifugation (5 h at 39000 r.p.m, in a SW41 rotor) on a linear (5 to 20%) sucrose gradient from CHO/pSWS cell lysates (10⁶ cells/ml). These fractions were incubated for 5 h at 37 °C with purified woodchuck serum albumin (prepared essentially as described by Machida et al., 1984) that had been polymerized by glutaraldehyde (as described by Yu et al., 1985) after having been coated for 18 h at 4 °C on microtitration plates (Falcon). Detection of WHsAg/WpSA complexes was attempted by using two different polyclonal antibodies directed against WHV S proteins (Shamoon et al., 1991), coupled to alkaline phosphatase. As shown in Table 2, we could not detect WHsAg bound to WpSA although the antibodies could clearly detect WHsAg-enriched fractions that had been directly coated on to microplates. In analogous conditions, an interaction between HBsAg particles purified from CHO/pSVS cell lines and HpSA was detected. HBsAg/HpSA complexes were also detected using both anti-WHV S antibodies which makes it unlikely that the failure to detect WHsAg/WpSA complexes was due to a deficiency of these detecting antibodies.

It should be noted that the in vitro derived WHV envelope particles contain little or no preS1 protein. However, the in vitro derived HBV envelope particles, which bind to HpSA, also have a deficiency in preS1
protein. The binding of viral particles to polymerized serum albumin therefore seems so far to be a phenomenon restricted to the human hepadnavirus. It remains unknown whether WHV and HBV share a common route of primary infection whereas binding to polymerized serum albumin may represent a secondary route of infection for HBV (Neurath et al., 1986; Pontisso et al., 1989), or may facilitate infection by the primary route.

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References


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