Cell type specific expression of pre S1 antigen and secretion of hepatitis B virus surface antigen

Brief Report

O. Marquardt1,*, K.-H. Heermann2, Maria Seifer2, and W.H. Gerlich2

1 Max-Planck-Institute of Biochemistry, Martinsried, and
2 Department of Medical Microbiology, Georg-August-Universität, Göttingen, Federal Republic of Germany

Accepted May 27, 1987

Summary

Production of the three hepatitis B surface (HBs) proteins was studied in a hepatoma cell line (PLC/PRF/5) and two HBs antigen secreting cell lines (HeLa and mouse L-cells), which had been transfected by a viral genome isolated by molecular cloning from PLC/PRF/5 chromosomal DNA. The DNA used for transfection contains the HBs-specific promoters and the enhancer which regulate the expression of HBs genes in the transfected cell lines. All three cell lines expressed well the small and middle HBs protein, but the larger pre S1 containing protein was barely detectable in the L-cell. In vivo growth of the transfected HeLa cell as nude mouse tumour increased pre S1 expression and suppressed secretion of HBsAg.

Hepatitis B virus (HBV) is one of the most important health risks in many regions of the world. Despite the lack of experimental systems to propagate it efficiently, much information on its structure and function has been achieved (reviewed in 25). The envelope of HBV is composed of small, middle and large proteins which share antigenic sites (HBsAg). They are all derived from one open reading frame (region S) which is translated either entirely or at start codons downstream. The products have common carboxy ends but differ in their 5' ends. Each of them is found in two forms distinguishable by the degree of glycosylation. According to the three start codons, region S is divided into pre S1, pre S2 and gene S.

HBV envelope proteins are also found in non-infectious filamentous particles and spherical HBsAg of 22 nm diameter. The non-infectious particles

are by far more abundantly produced than virions. Analysis of the protein composition of infectious and non-infectious particles revealed i) comparatively lower amounts of pre S 1 proteins in 22 nm-particles than in virions and filaments and ii) a high proportion of HBs small proteins in all particles (8). Such prevalence corresponds with abundant transcription specific for small and middle proteins as reported for liver of HBV-infected chimpanzees (1), the naturally HBsAg-producing human hepatoma cell line (PLC/PRF/5) (18), and cell lines which produce HBsAg following transfection with HBV-DNA (5, 7, 21, 22). RNA which could account for the translation of large protein, however, is rare or not found in these cells.

Whether large HBs proteins are produced by a given system should not only be deduced from transcription analyses alone but should also be analyzed at the protein level, e.g. by the use of monoclonal antibodies. Such were available to us; the monoclonal antibody MA 18/7 (8) recognizes a pre S 1 domain, and the monoclonal antibody Q 19/10 (11) recognizes the glycosylated pre S 2 domain. We were thus able to carry out a comparative study on the production of the three surface proteins in three different cell lines which harbour one and the same HBV DNA. Cells were i) the human hepatoma cell line PLC/PRF/5 (12) which produces HBsAg due to constitutive expression activity of integrated HBV genes; ii) the murine L-cell clone LTK/HBs/2425 (5) which produces HBsAg following transfection with a HBV-specific HindIII genome fragment of PLC/PRF/5 cells of 10.7 kilobases (9). The clone was kindly provided for these studies by Dr. Freytag v. Loringhoven. iii) The HeLa/HBs/B 14 clone obtained following transfection of standard HeLa cells by the 10.7 kilobase DNA mentioned above together with the plasmid pSVgpt (16) according to the protocol of Graham and van der Eb (6). These are the details of the transfection experiment with the HeLa cells: The HBV-specific DNA was linear and added in 3-fold molar excess over the selection marker. Selection of transfected cells was carried out in Eagles Medium modified by the addition of xanthine (250 μg/ml), hypoxanthine (15 μg/ml), additional L-glutamine (150 μg/ml), thymidine (10 μg/ml), aminopterin (2 μg/ml), mycophenolic acid (25 μg/ml), kanamycin (50 units), and fetal calf serum (10 per cent). Three weeks post transfection 20 clones were picked with cloning cylinders and transferred to culture wells (Costar). Five clones were found to secrete HBsAg. Four of them produced continuously HBsAg, however at different titers. Clone 14 produced the highest HBsAg titer and has therefore been chosen for further studies. HBsAg was detected by the AusriaII-125 test kit (Abbott Laboratories, North Chicago, Ill., U.S.A.).

The production of the three different surface proteins by different cells was analyzed by several methods. For ELISA, purified monoclonal antibodies (1 μg/ml) were used to coat microtiter plates (Nunc II) in 0.02 M phosphate buffer pH 7.4. After post-coating with 1 per cent bovine serum