Hepatitis B Virus-Mediated Changes of Apolipoprotein mRNA Abundance in Cultured Hepatoma Cells

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An inverse correlation between hepatitis B virus (HBV) and steady-state levels of apolipoprotein AI and CIII mRNAs was observed in two hepatoma cell lines. Analysis of a third line containing an inducible viral genome implicated viral pregenomic RNA in apolipoprotein mRNA reduction. We conclude that HBV alters infected cells despite the absence of overt cytopathogenicity.

Chronic infection with hepatitis B virus (HBV) greatly increases the risk for developing liver cirrhosis and hepatocellular carcinoma (1, 2). HBV infection is generally considered to be noncytopathic, as chronic viremia is not accompanied by signs of hepatocyte damage. Instead, considerable evidence points to an important immune and inflammatory contribution to liver dysfunction (5). However, as virus pathology remains incompletely understood, it is possible that the replication of HBV and/or expression of viral gene products affects hepatocellular function at the molecular level. For example, the HBV transactivator, HBx, can activate a number of cellular genes (9).

To probe for previously unrecognized interactions between HBV and host cells, RNA was isolated with Trizol (Invitrogen Life Technologies) from human HepG2 hepatoblastoma cells (American Type Culture Collection) and subline HepG2.2.15 cells. HepG2.2.15 cells are stably transfected with a complete HBV genome; they express all viral RNAs and proteins, produce viral genomes, and secrete virus-like particles (15). DNA microarray analysis comparing the transcriptional profiles of the two cell lines was performed by a commercial service (Merged, Ltd.). Of 1,059 genes queried on array H02, 72 mRNAs were up-regulated by twofold or more in the HepG2.2.15 cells and 87 were down-regulated. The latter group included multiple apolipoprotein genes normally expressed by hepatocytes; those apolipoprotein genes that produced signal above background in at least one of the two cell types are listed in Table 1. Apolipoprotein AI (apoAI) represents the major protein component of high-density lipoprotein (HDL), and apoB represents the major protein constituent of very-low-density lipoprotein; both are normally secreted by hepatocytes. As HBV can be considered a lipoprotein particle itself, disruption of either type of normal lipoprotein secretion would represent an unusual host cell response to chronic viral infection.

RT-PCR was used to verify the down-regulation of selected liver-enriched apolipoprotein genes in HBV-expressing cells; apoCIII mRNA levels were also examined, as the apoCIII gene is adjacent to the apoA1 gene and they share certain regulatory elements (19). Reverse transcription (RT) was performed with 2 μg of total RNA and 50 ng of random hexamer primers (11). Five to 10% of each cDNA was used as a template in PCRs using gene-specific primers (sequences are available upon request) and Taq DNA polymerase (Stratagene). The number of cycles needed for each primer set to ensure that amplification was within the linear range was established empirically. PCR products were separated by electrophoresis through 2% agarose or 5% nondenaturing polyacrylamide gels and detected by ethidium bromide staining. Images were captured and relative quantitations were made with an Alphalmager digital imaging system (Alphalmimotechn). Initial experiments confirmed the general trend of apolipoprotein mRNA reductions in HepG2.2.15 cells relative to levels in HepG2 cells (Fig. 1a). However, the expression of viral genes in HepG2.2.15 cells is fairly low unless the cells are held at confluence for several days or more (15), which may account for the magnitude of the difference in apolipoprotein mRNA levels determined by RT-PCR versus microarray analysis.

To investigate a possible inverse correlation between apolipoprotein mRNA levels and HBV levels, RNA was prepared from HepG2.2.15 cells maintained at confluence for increasing amounts of time. Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum was replaced every other day to maintain cell viability. RT-PCR analysis revealed that levels of

<table>
<thead>
<tr>
<th>Gene</th>
<th>HepG2 cells</th>
<th>HepG2.2.15 cells</th>
<th>Change (n-fold)</th>
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<tbody>
<tr>
<td>apoAI</td>
<td>10,797.7</td>
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<td>apoAII</td>
<td>31,458.1</td>
<td>2,307.3</td>
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<td>apoB</td>
<td>16,948.0</td>
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<td>7,483.4</td>
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<td>apoCII</td>
<td>4,856.2</td>
<td>1,323.2</td>
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<tr>
<td>apoCIII</td>
<td>692.8</td>
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<tr>
<td>apoH</td>
<td>17,679.9</td>
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<td>64.0</td>
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<tr>
<td>apoM</td>
<td>4,057.2</td>
<td>128.4</td>
<td>31.6</td>
</tr>
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</table>

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* Raw pixel values were normalized for overall signal intensity across the two arrays, and local background values were subtracted. Values of 1.0 are equivalent to background. Data shown are from a single comparison; a duplicate experiment gave similar results.
apoAI and apoCIII mRNAs declined strikingly with increasing time in culture; apoB mRNA levels declined slightly (Fig. 2). In contrast, levels of viral pregenomic RNA increased over the same time period by a factor of ca. 10-fold. Actin mRNA levels remained constant during the first 7 days but declined by day 14. Transcription of cellular genes was not repressed globally, as no change was observed in β9251-antitrypsin mRNA abundance; this finding also suggests that hepatocyte-specific gene expression is not generally repressed. Thus, levels of apoAI and apoCIII mRNAs correlated inversely with levels of virus. Incubation of HepG2 monolayers for 7 or 14 days did not result in any change in apoAI mRNA levels (data not shown), demonstrating that the changes observed with HepG2.2.15 cells were not due to culture age or density. The reason for the discrepancy between the microarray and RT-PCR results for apoB is not known.

To strengthen the link between viral replication and apolipoprotein gene expression, a second cell pair was studied. Q7-21 cells were derived by transfection of a McA-RH7777 hepatoma cell subline with a full-length HBV genome (16). Unlike HepG2.2.15 cells, Q7-21 cells express a high level of viral gene products regardless of cell density. RNA was isolated from confluent cultures of McA-RH7777 hepatoma cells (American Type Culture Collection) and virus-containing Q7-21 cells. RT-PCR analysis demonstrated that levels of apoAI and apoCIII mRNAs were reduced in the virus-producing cells relative to the level in uninfected parental cells (Fig. 1b). The level of apoB mRNA was also reduced, in contrast to the minimal effect seen with the HepG2.2.15 cells (Fig. 1a and 2). The two cell types differ in species, gender, and HBV subtype (adw2 for 2.2.15; ayw for Q7-21). In addition, HepG2 cells secrete apoB in abnormal particles that resemble LDL rather than very-low-density lipoprotein (17), which might affect the response of the apoB gene to regulatory cues.

Concern remained that selection of cells capable of supporting virus replication and gene expression also selected for cellular changes that coincidentally reduced apolipoprotein gene expression. HepG2 AD38 cells contain a complete HBV genome under the control of a tetracycline-off promoter (13). In the presence of tetracycline, these cells express low levels of the 3.5-kb pregenomic RNA that also serves as the mRNA for core protein and polymerase. The drug does not suppress the 2.5- and 2.1-kb surface glycoprotein transcripts, as they derive from transcription initiating at the internal pre-S1 and S promoters (Fig. 3a).

RNA was isolated from HepG2 AD38 cells maintained in

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**FIG. 1.** Comparison of apolipoprotein mRNA levels in cells that do or do not contain replicating HBV. (a) Relative levels of apoAI, apoB, apoCIII, and actin mRNAs present in HepG2 and HepG2.2.15 cells were compared by RT-PCR. Amplified products were separated by gel electrophoresis and detected by staining with ethidium bromide. The identity of each product was confirmed by DNA sequencing. (b) Relative levels of apoAI, apoB, apoCIII, and actin mRNAs present in McA-RH-7777 and Q7-21 cells were compared by RT-PCR. Rat-specific primers were used for apoB and apoCIII; the human-based primers for apoAI and actin were capable of detecting the rat homologues. The identities of the amplified rat products were confirmed by DNA sequencing.

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**FIG. 2.** Inverse correlation between viral RNA levels and apolipoprotein mRNAs. Total RNA was prepared from cells incubated at confluence for increasing lengths of time, so as to yield increasing levels of viral nucleic acids and protein. (a) RT-PCR was used to detect HepG2.2.15 cell mRNAs for apoAI, apoB, apoCIII, α1-antitrypsin (AAT), actin, and viral RNA (HBV). Note that HBV primers specifically detect the pregenomic RNA and not the subgenomic mRNAs. The image contrast was reversed to improve the visibility of faint bands. (b) Plot of densitometric data illustrating the relative changes in steady-state levels of mRNAs over time in culture.
Dulbecco’s modified Eagle’s medium plus 10% fetal calf serum and tetracycline (Fig. 3b, day 0 with tetracycline). AD38 cells were seeded in triplicate and incubated in the absence of tetracycline for 2, 4, or 6 days prior to RNA isolation. A parallel culture was maintained for 6 days in the presence of tetracycline prior to RNA isolation to control for cell density and culture age effects. As expected from the published report, HBV pregenomic RNA was induced by 4 and 6 days following tetracycline withdrawal. The time-dependent reduction in apoAI and apoCIII mRNAs declined following tetracycline withdrawal. The pregenomic RNA in apolipoprotein mRNA reduction, also include effects of infected hepatocytes in important ways despite lacking overt cytopathogenicity.

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REFERENCES


