Hepatitis B Virus DNA Is Enriched in Polymorphonuclear Leukocytes

By D.I. Hoar, T. Bowen, D. Matheson, and M.C. Poon

DNA hybridization and cell separation techniques were used to determine which blood components contained hepatitis B viral DNA sequences. Free monomer-length hepatitis B virus was found in large amounts in the polymorphonuclear leukocyte cell fraction in two of five HBsAg-positive patients. In these two patients, viral DNA sequences were not detected in the plasma or platelet fraction, whereas the mononuclear cell DNA contained small amounts of a 7.2 kb size unintegrated hepatitis B genome. These studies indicate that the major reservoir of unit-length viral DNA in the asymptomatic hepatitis B carriers studied here was in the polymorphonuclear leukocyte fraction. The basis for the presence of the viral DNA within these cells is presently unknown, but may relate to viral replication within, or phagocytosis of virus by, these cells.

HEPATITIS B virus (HBV) has classically been considered an hepatotropic virus; however, blood components from HBsAg-positive patients are capable of transmitting hepatitis B, indicating that some or all components of peripheral blood contain infective HBV. Two recent studies have identified the presence of the HBV genome in mononuclear cells and white blood cells of some HBsAg-positive individuals. Although there is little correlation between specific serological findings and the presence of the viral genome in a free or integrated state within mononuclear cells, HBeAg or Anti-HBe seems to be present in all patients with HBV genome in any state.

We have used Southern transfer and hybridization using cloned HBV genome to study the status of the HBV genome in separated components of the blood from chronic HBV carriers in an effort to identify blood component sources of HBV DNA and hence predict the infective component(s). Two of five HBsAg-positive individuals exhibited specific enrichment of monomer-size HBV DNA within their polymorphonuclear (PMN) cell fraction.

MATERIALS AND METHODS

Patients. Serological investigation of five asymptomatic chronic HBV carriers was undertaken for HBsAg, HBeAg, and Anti-HBe using commercial solid-phase radioimmunoassay (Abbott Laboratories). Control samples known to be negative for HBsAg, HBeAg and Anti-HBe were obtained from healthy blood donors.

Isolation of DNA from blood components. After obtaining informed consent, heparinized peripheral blood was collected and processed within four hours, using mono-poly resolving medium (M-PRM) (Flow Laboratories, Mississauga, Ontario, Canada). Seven milliliters of whole blood was underlaid with 6 mL of M-PRM and centrifuged at 1,200 g for ten minutes at room temperature. Platelet-rich plasma, mononuclear (MN), and PMN fractions were removed and washed by centrifugation (600 g) for five minutes) twice with normal saline (the M-PRM separation used in these studies results in MN with 4.9% to 8.3% PMN and a PMN fraction with 2.9% to 3.7% MN). Washed component fractions were resuspended in 40 mmol/L of Tris pH 8.0, 40 mmol/L of EDTA (TE), lysed with an equal volume of 0.2% sodium dodecyl sulfate (SDS) in TE, and DNA was extracted. Total DNA was also isolated from whole blood according to published techniques. These procedures yield DNA with <10% RNA contamination when they are used on blood components. For the serum fraction, salmon sperm DNA was added to serum (4 µg/100 µL of serum), and the DNA was extracted immediately following lysis with 0.2% SDS in TE.

DNA analysis. Purified nucleic acid samples were resuspended in 40 µL of Tris pH 8.0 and 0.4 mmol/L of EDTA, and the concentration was determined by reading the optical density at 260 nm. Aliquots of 1.0 and 0.1 µg were dotted onto dry nitrocellulose (Schleicher and Schuell Keene N.H. BA-83) that had been previously soaked in 3 mol/L of sodium chloride and 0.3 mol/L of sodium citrate (20X SSC).

Standards of cloned HBV DNA at concentrations of 1 to 5,000 pg and pBR322 standards of 1 to 100 ng were dotted on each filter. After the DNA dots had dried thoroughly, the nitrocellulose was floated three times for one minute on 0.2 N NaOH, 1.5 mol/L of NaCl (blotting briefly between each) and three times for one minute on 2X SSC, 0.2 mol/L of Tris pH 7.5, and 4 mmol/L of EDTA, and baked at 80 °C in a vacuum oven prior to hybridization.

Restriction digestion of DNA, Southern transfers, and hybridization procedures were as previously described except that the 32p-labeled probe used was the total cloned HBV genome purified from the vector pBR322 following EcoRI digestion. The specific activities of all probes were >2 x 105 cpm µg, and were used at a concentration of 1 to 2 x 104 cpm/µL.

RESULTS

Dot hybridization analysis of control samples from six individuals failed to reveal any hybridization. Two HBsAg-positive carriers that were also Anti-HBe positive exhibited the presence of significant viral genome; this was enriched in the PMN fraction (Table I). Figure 1A shows the results of Southern hybridization analysis of 20 µg of undigested DNA from the PMN fractions of the HBsAg-positive patients 1, 2, 3, and 5 (lanes 1, 2, 3, and 5, respectively) and the corresponding MN fraction from patient 5 (lane 4). The major hybridization in lanes 3 and 5 at about 3.2 kilobases (kb) corresponds to free HBV DNA. There are also higher mol wt forms hybridizing at about 7.2 kb. In Fig 1B, 10-µg samples of MN and PMN DNA from patient 5 were analyzed undigested or following EcoRI digestion. In addition to the 3.2-kb free HBV DNA in the PMN fraction, a faint band is evident at about 7.2 kb in both the digested and undigested PMN fraction and in the undigested MN fraction. Similar results were obtained using HindIII. Undigested, EcoRI, digested, and BamHI digested samples (1 µg)


Table 1. Results of Dot Hybridization

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<th>HBsAg</th>
<th>eAg</th>
<th>Anti-</th>
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Hybridization analysis performed on 1.0 μg total cellular DNA or DNA extracted from 100 μl of serum containing 4 μg carrier salmon sperm DNA. Sensitivity was 0.5 pg HBV genome; + = <1.5 pg; ++++ = >50 pg.

MN, mononuclear cell fraction; PMN, polymorphonuclear cell fraction.

of the PMN fraction from patient 5 were analyzed by gel electrophoresis (Fig 2); the results gave the expected HBV genome bands of ~1,450, 900, 475 and 280 base pairs, indicating the existence of a major component of free HBV DNA within this sample (the smallest fragment is not evident on this exposure).

DISCUSSION

In this study of peripheral blood from five HBsAg-positive patients, we have demonstrated in two patients that HBV unit-length DNA is found predominantly in the PMN leukocyte fraction of blood. There is evidence of a very small amount of HBV DNA in the mononuclear fraction of these patients (<10% of that observed in PMN DNA). This DNA is larger than the 3.2-kb unit length (~7.2 kb) and does not appear to be integrated, as it is evident in undigested DNA (Fig 1B). This DNA must be cell-associated, as each blood component fraction was washed prior to lysis and DNA extraction. In addition, the marked unit-length HBV hybridization found in PMN DNA and absent from MN or platelet DNA or serum argues against HBV DNA contamination from serum. In the study by Lie-Injo et al, total white cell

Fig 1. (A) Southern hybridization analysis of 20-μg aliquots of undigested white cell DNA from patients 1 through 4. Lanes 1, patient 1 polymorphonuclear cell fraction (PMN); 2, patient 2 PMN; 3, patient 3 PMN; 4, patient 5 mononuclear cell fraction (MN); 5, patient 5 PMN. The undigested DNAs were separated on a 1% agarose gel and then were transferred and hybridized with HBV probe at 4 x 10⁶ cpm per microgram, 2 x 10⁶ cpm per milliliter. Autoradiography was for 16 hours; marker sizes (left) are in kilobases (kb). (B) Autoradiograph of Southern transfer of MN and PMN DNA from patient 4. Lane 1, MN undigested; lane 2, PMN undigested; lane 3, MN EcoRI digested; lane 4, PMN EcoRI digested. Autoradiography was for 24 hours; conditions otherwise were as in A.

Fig 2. Southern hybridization analysis of PMN DNA from patient 5. One-microgram aliquots were run undigested (A); EcoRl digested (B); and BamHI digested (C) on a 1% agarose gel; the aliquots were then Southern transferred and hybridized to a HBV probe at 3 x 10⁶ cpm per microgram, 10⁶ cpm per milliliter. DNA marker sizes are given in kb (left).
DNA preparations were used; hence, the source of HBV DNA cannot be determined. The studies of Pontisso et al\textsuperscript{2} using Ficoll-Hypaque-separated MN indicated HBV presence; however, data on PMNs was not discussed. The basis for the differences between this previous study and our present study is unclear; however, different cell fractionation methods were used.

The observed PMN fraction enrichment of monomer HBV DNA could be the consequence of at least four, not necessarily mutually exclusive, events. In the first instance, HBV may be replicating within the PMNs or a specific subset of them; thus, the PMN separation would be expected to enrich for HVB monomer, and it would not necessarily appear in the MN fraction. A second possibility is that replication of HBV within a MN cell could cause a change in the density, resulting in its separation from the MN fraction and shift to the PMN fraction. A third and equally likely possibility is that a phagocytic PMN is scavenging either HBV or HBV-infected cells, resulting in the PMN enrichment. A final possibility is that the HBV DNA is only extracellular and in viral particles adherent to cells within the PMN fraction.

The data presented here do not permit a resolution of the above possibilities; however, our data clearly demonstrate that in some chronic carriers of HBsAg, the viral genome is enriched in the PMN fraction.

REFERENCES

3. Lie-Injo LE, Balasegaram M, Lopez CG, Herrera AR: Hepatitis B virus DNA in liver and white blood cells of patients with hepatoma. DNA 2:301, 1983
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