Chromosome Abnormalities in Peripheral Blood Cells of Hepatitis B Virus Chronic Carriers

Daniela Simon, Thomas London, Hie-Won L. Hann, and Barbara B. Knowles


ABSTRACT

Cytogenetic analysis of metaphase chromosome spreads from peripheral blood cells of hepatitis B virus (HBV) chronic carriers and HBV-negative individuals of the same ethnic origin revealed a significantly higher incidence of chromosome breaks and other mitotic aberrations in the HBV chronic carriers. The highest incidence of chromosome breaks was found in chronic carriers who evidenced circulating HBV. Such an association between HBV and these genetic lesions assumes importance in light of the known correlation between HBV chronic carrier status and the high risk of hepatocellular carcinoma, where a mutagenic effect of HBV cannot be excluded.

INTRODUCTION

HBV infection and development of the chronic carrier state is strongly correlated with a high incidence of PHC (1, 2). Although HBV integrates in the host genome, none of the HBV gene products has proven to have direct transforming potential, no common site of HBV integration in the multiple tumors examined has been identified (3), and no underlying molecular defect common to each PHC has been documented. The role of HBV in PHC still remains enigmatic. One possible effect of HBV on the infected cells is induction of random mutations. This possibility has not been investigated because of the limited experimental systems available. We previously found chromosome aberrations in metaphase chromosomes from peripheral blood cells of an HBV chronic carrier with hepatocellular carcinoma (4). In light of the results of others who reported that HBV could infect and replicate in populations of lymphoid cells (for review, see Refs. 5 to 13), we embarked on a pilot study to determine the level of chromosome abnormalities in peripheral blood cells from a group of Asian immigrants, some of whom were HBV chronic carriers. We now report differences in the proportion of chromosome and other mitotic aberrations within this population. HBV chronic carriers have a significantly higher incidence of abnormalities; those who contain detectable circulating vihros have the highest proportion of metaphase chromosome spreads with chromosome breaks. These data suggest that HBV infection might have a more generalized effect on chromosome stability than previously recognized.

MATERIALS AND METHODS

Source of Human Material. Blood samples, obtained from 40 individuals, were screened for HBV carrier status at the Liver Cancer Prevention Center at the Fox Chase Center, Philadelphia, PA. This center seeks to serve as a registry and early warning center for those at risk for the development of liver tumors. The 40 participants in the study are immigrants from East Asian countries, including Korea, China, Taiwan, and Vietnam and are between 18 and 58 yr of age. Thirty-one were classified as HBV carriers based on persistence for more than 6 mo of seropositivity for HBsAg. It is likely that all of the carriers were infected in their native countries during infancy or childhood. Nine individuals were HBsAg negative and HBV-DNA negative and were considered free of current HBV infection; one of these nine had anti-HBs and another had anti-HBe, indicating their past acute infection with HBV (Table 1). Of the 31 HBV carriers, 13 were female and 18 were male; of the 9 uninfected persons, 4 were female and 5 were male. None of the 40 subjects had a history of exposure to radiation or mutagenic chemicals, all were regular tea drinkers, and of those who were smokers none smoked more than one-half package per day. Seven of the HBV chronic carriers reported a close relative who had succumbed with hepatocellular carcinoma.

Serum Assays. Each serum sample was tested for the presence of major HBV antigens and antibodies (HBsAg, anti-HBs, HBeAg, anti-HBe, and antibodies to HBcAg), using commercial enzyme-linked immunosorbent assays (Abbott Laboratories, North Chicago, IL) according to the manufacturer's instructions. All positive tests were repeated and confirmed using the manufacturer's confirmation procedures.

Chromosome Analyses. Chromosome analysis was performed, without knowledge of the HBV status or clinical history of the subjects, by standard methods after a 72-h incubation of whole blood at 37°C in DMEM.10 and phytohemagglutinin (0.1 µg/ml; Wellcome Diagnostics, Dartford, England). Between 20 and 40 metaphase chromosome spreads from each individual were analyzed by the same worker and scored for chromosome breaks and other mitotic aberrations, such as pulverization or premature chromosome condensation, premature centromere divisions, triradials, telomeric breaks and minute chromosome endoreduplication, and hyperploidy (Fig. 1). Chromosome preparations were analyzed after staining in 1% Giemsa or after treatment by G- and C-banding techniques (14, 15).

DNA Extraction and Southern Blot Analysis. Peripheral blood cells were separated from plasma by centrifugation (1500 × g, 10 min) and washed 3 times in DMEM.10. Both plasma and cells were treated with proteinase K (200 µg/ml) in a solution of 0.01 M Tris-HCl (pH 7.4), 0.001 M EDTA, 0.1 M NaCl, and 0.5% sodium dodecyl sulfate for 18 h at 37°C. DNA was extracted by the standard phenol:chloroform procedure, precipitated with ethanol, and solubilized in 0.01 M Tris, 0.001 M EDTA, pH 7.4. Cellular DNA was digested with HindIII, according to the manufacturer's specifications (New England BioLabs, Beverly, MA), separated on a 0.8% agarose gel, and blotted onto Nytran (16). Filters were hybridized with a 32P-labeled nick-translated full-length HBV insert (17), purified from pBR322 sequences by electrophoresis and elution.

Statistical Analysis. The frequency of the various chromosome abnormalities in each individual was calculated, groups made, ranks assigned, and groups compared by the Mann-Whitney U test, using a correction for ties (18). The significance of the presence or absence of abnormalities between groups were tested by the Fisher exact probability test (18). For purposes of statistical analysis, chromosome breaks were considered together as possible evidence of direct genetic damage, while all of the other mitotic abnormalities were grouped together.
CHROMOSOME ABNORMALITIES IN PERIPHERAL BLOOD CELLS

Table 1 Clinical status of individuals within the population investigated

<table>
<thead>
<tr>
<th>HBV DNA</th>
<th>HBSAg</th>
<th>HBeAg</th>
<th>HBSAg</th>
<th>HBeAg</th>
<th>HBCAg</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV chronic carriers</td>
<td>31</td>
<td>18</td>
<td>31</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>HBV negative</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

* Southern blot hybridization.
* All individuals with circulating HBV contained HBeAg.

RESULTS

The chromosome analyses were performed and evaluated without knowledge of the patient’s history. The incidence of chromosome breaks and other mitotic aberrations was observed (Fig. 1). Correlation of the chromosome analyses with the clinical status of the individuals revealed a significantly higher frequency of metaphase chromosome spreads containing abnormalities in HBV chronic carriers than in the HBV-negative individuals (Fig. 2). Sixteen of the HBV chronic carriers exhibited a higher frequency of metaphases containing abnormalities than any of the HBV-negative individuals investigated. On average, more than 20% of the cells analyzed from the HBV chronic carriers contained abnormalities (Table 2). When the frequencies of all aberrations were ranked and the groups compared by the Mann-Whitney U test, a highly significant difference was found between the HBV chronic carrier and HBV-negative individuals (z = 2.73; P = 0.003). Partitioning the abnormalities revealed a highly significant difference between these two populations in the incidence of chromosome breaks (Fisher’s exact probability = 0.002) and also other chromosome abnormalities (Mann-Whitney U test; z = 3.2, P = 0.0007). Although there was an elevated level of all categories of abnormalities, the most obvious finding was that of single minute chromosomes in multiple copies in numerous metaphase spreads.

Eight of the 31 HBV chronic carriers contained readily detectable levels of HBV DNA in their plasma by Southern blot analyses. Fig. 3 shows a representative Southern blot analysis of DNA extracted from the plasma and peripheral blood cells of two carriers. The HBV genome was detected in the plasma as either a discrete 3.2-kilobase band representing the intact HBV genome or as a smear of hybridization beginning at 3.2 kilobases, a pattern observed during active viral replication. Analysis of the metaphase spreads from the HBV chronic carrier population revealed that the incidence of chromosome breaks was significantly higher in the peripheral blood cells of those HBV chronic carriers with detectable circulating HBV DNA than in the remaining chronic carriers (Table 3, Fisher’s exact probability = 0.03). We found no significant difference in the frequency of the other mitotic aberrations in chronic carriers with or without circulating levels of HBV (z = 0.541; P = 0.29). Thus, the presence of a high level of circulating virions correlates with a relatively high frequency of direct chromosome damage but not with an increased frequency of other mitotic abnormalities in the metaphase chromosome spreads.

DISCUSSION

In the present study, we describe a highly significant difference in the frequency of mitotic abnormalities in metaphase spreads of peripheral blood cells from HBV chronic carriers.

Fig. 1. Representative chromosome aberrations in metaphase spreads in this study. A, normal; B, breaks and gaps (arrows); C, premature chromosome condensation or pulverization (arrow); D, triradial formation (arrow); E, endoreduplication; F, minute chromosomes (arrow); G, premature centromere division; and H, hyperploidy.
when compared with those from peripheral blood cells of HBV-negative individuals. The large number of minute chromosomes in the HBV chronic carriers is quite striking, although their origin and significance are currently unclear. The only previous report of minute chromosomes in peripheral blood cells of seemingly normal individuals was from a population of South American Indians (19) whose use of hallucinogenic drugs was considered a possible generative factor. However, the appearance of minute chromosomes proved ephemeral and the cause, be it substance abuse or exposure to a toxic or an infectious agent, was never established (20).

The correlation between an increased frequency of chromosome aberrations and the HBV carrier state and the fact that those chronic carriers with detectable HBV in the plasma exhibited the highest frequency of chromosome breaks suggest that HBV may have a role in their induction. Chromosome lesions, such as the fragmentation that occurs upon measles virus infection, have long been associated with virus-infected cells (21). In the only published study of metaphase chromosome spreads of peripheral blood cells of patients in the acute phase of infection with hepatitis A and B viruses, an elevated level of chromosome aberrations and of sister chromatid exchanges was revealed (22). We now show that chronic HBV infection is also associated with chromosome abnormalities. Chromosome damage may represent a direct or indirect effect of HBV integration or replication. In hepatoma cells integrated HBV sequences are frequently associated with rearrangements of the host flanking sequences detectable at the molecular level (e.g., see Ref. 23).

Chromosome studies of normal or HBV-infected hepatocytes have not been reported. However, even though HBV replicates in some cells of hematopoietic origin (5–13), the hepatocyte is the major reservoir of HBV infection. Typically many years of persistent HBV infection precede hepatocellular carcinoma, a period during which chromosome damage may occur and mutations accumulate. Liver damage by the antiviral immune response and compensatory regenerative proliferation may set the stage for selection of those mutations which deregulate liver cell growth, thus providing a basis for hepatocellular carcinogenesis.

**REFERENCES**


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