Selective Transmission of Hepatitis B Virus after Percutaneous Exposure

Siew L. Ngui,1,a Ruth P. F. Watkins,2 Julia Heptonstall,3 and Chong G. Teo1

In 3 clusters of postsurgical hepatitis B virus (HBV) infection, HBV DNA sequence mismatches were observed between the transmitting surgeons and the patients whom they infected. Sequence analysis of clones amplified from the C gene of HBV suggested that the mismatches were due to transmission of a minority variant in the circulation of each surgeon. Compared with 5 other transmitters from whom transmission of the dominant variant was demonstrated, the 3 surgeons who transmitted minority variants carried significantly more heterogeneous HBV populations. Transmission of minority variants was not correlated with the transmitters’ hepatitis B antigen status, the presence of the position 1896 precore mutant, or the level of HBV viremia. In 1 cluster, a variant comprising <10% of the HBV population circulating in the transmitting surgeon established infection in all 3 patients who acquired HBV through him, which substantiates the phenomenon of true selection.

Hepatitis B virus (HBV) can be transmitted nosocomially: from patient to patient [1–9]; from patient to healthcare workers (HCWs) [10, 11]; and from HCWs to patients [12–18]. Transmissions associated with infected HCWs are under close surveillance in England and Wales [19, 20]. A series of postsurgical outbreaks in the 1980s and early 1990s [12] prompted the United Kingdom Health Departments to issue guidance aimed at minimizing the risk of HBV transmission to HCWs and patients [16]. The guidance recommended that HCWs in whom hepatitis B antigen (HBeAg) is serologically detectable should not perform exposure-prone procedures. HBV-infected HCWs who are HBeAg-negative may continue to practice, unless they have been shown to be associated with transmission. However, a number of transmission incidents associated with HBeAg-seronegative carrier surgeons have been reported [21, 22]. Such outbreaks are associated with HBV variants with mutations in the precore region of the genome that interfere with the production of HBeAg [23].

Currently, molecular epidemiologic investigations of HBV transmission are polymerase chain reaction (PCR)–based [24–26]. Compared with RNA viruses, HBV mutates relatively slowly [27], allowing, in most transmission incidents, perfect matching between sequences of HBV subgenomic fragments amplified from samples from the source and those amplified from the cases. While investigating HCW-associated transmissions between 1990 and 1997, we observed in some incidents that subgenomic sequences carried by HBV of the implicated transmitter and by the cases, although phylogenetically related, were different. Such occurrences pointed to selective HBV transmission. Here we describe investigations that substantiate this phenomenon and correlate selective transmission with characteristics of the virus carried by the transmitter.

Methods and Materials

Samples. The provenance of serum specimens from 8 HCWs and the related transmission events are described in table 1. For the present study, sera from 26 cases of acute hepatitis that were linked epidemiologically to the HCWs were used.

Detection of HBV DNA. HBV DNA was extracted from 50 µL of each serum sample by use of a guanidinium thiocyanate and silica particle–based method [23]. The HBV DNA was amplified by nested PCR, by use of primer sets targeting a 263-bp fragment of the core gene [25].

Single-strand conformation polymorphism (SSCP) assay. Both surface and core amplicons were submitted to a previously described SSCP analysis [21]. In brief, 32P-labeled second-round PCR products were denatured, then electrophoresed through a nondenaturing gel, following which radioactive bands were detected by autoradiography.

Cloning of PCR products. Amplicons generated from the sera of the HCWs were cloned into the vector and transformed in competent cells provided in the LigATor kit (R&D Systems, Minneapolis, MN).

DNA sequencing. Amplicons were purified by use of GeneClean (Bio101, Vista, CA) and sequenced by use of the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (PerkinElmer, Foster City, CA) and an Applied Biosystems 373A automated sequencer (Foster City, CA). The data generated were an-
analyzed by use of Analysis and SeqEd (Applied Biosystems) and Lasergene Navigator (DNASTAR, Madison, WI).

Precore mutation detection. The HBV DNA from the HCWs was examined for the presence of the precore mutation at nucleotide 1896 by use of the amplification-created restriction-site method described by Lindh et al. [28].

Estimation of viral load. The viral load within the HCWs was semiquantified by use of end-point dilution PCR. Each specimen was diluted 10-fold and the end-point compared with that of 1 ng of the plasmid diluted in a similar manner.

Evaluation of HBV quasispecies heterogeneity. The extent of quasispecies heterogeneity in each transmitter’s specimen was measured in 2 ways. In the first, the proportion of HBV clones not bearing the dominant (majority) sequences was calculated to give what is described here as the heterogeneity index. In the second, Shannon entropy analysis [29] was applied. This entropy measure was calculated as \( S = -\sum (p_i \ln p_i) \), which took into account the frequency of each sequence \( p_i \) in a given quasispecies. The entropy value was then normalized as \( S_n = S / \ln N \), to take into consideration the total number of sequences \( N \) analyzed in each quasispecies.

Results

Direct sequences derived from the HBV core fragment were compared with each other and with sequences from GenBank by use of the Clustal method of sequence alignment. The sequences from each incident segregated into distinct clusters. In 5 of the clusters (A, B, C, D, and E), the implicated HCW and related patients possessed sequences that were indistinguishable by SSCP analysis (representative pictures are shown in figure 1). This was confirmed by DNA sequencing (data not shown). In 3 other clusters (F, G, and H), the sequences amplified directly from the HCW and their patients, although closely related, were different (figure 2). For cluster F, the sequence in the case differed from the transmitter by a single base, at position 1975, which led to a ser\(^{\text{g}}\)-phe change. For cluster H, there were 3 base changes, at positions 2071, 2073, and 2092; these were all nonsynonymous (his\(^{\text{g}}\)-gly, val\(^{\text{a}}\)-ala, and asp\(^{\text{g}}\)-glu, respectively).

For each HCW, amplicons derived from the core gene subfragment were cloned, and then their sequences were compared with each other and with the sequence directly amplified from the patients. The results are summarized in table 2. The direct sequence from the patient was identical to that of a proportion of clones from the HCW. However, the proportion varied between clusters A–E as one group and F–H as another group. For clusters A–E, the sequences of 59%–80% of clones derived from HCWs; clusters A–E were identical to the direct sequence from the HCW and to that from their respective patients. For HCW F, none of the cloned sequences were identical to the sequence directly amplified from the transmitter’s serum, and only 20% of the clones were identical to the patient’s sequence. The clones recovered from HCWs G and H showed a similar range of heterogeneity: 11% and 33% of the clones, respectively, were identical to their direct sequences, whereas only 6% and 7%, respectively, were identical to the patient’s sequence.

The characteristics of transmitters in the group in which transmission of the dominant HBV variant was observed (clusters A–E, group I) were compared with those in the group in which transmission of minority variants was observed (clusters F–H, group II). The results are summarized in table 3. There were no significant differences between transmitters in their HBeAg status, the presence of the 1896 precore mutant, or the level of viremia. However, the heterogeneity of DNA sequences in the PCR clones derived from the C gene of transmitters, as measured by the heterogeneity index and by entropy calculations, was different.
Discussion

Transmissions of minority HBV variants have been reported after perinatal infection [30]. Selective transmission has also been observed for other viruses (e.g., human immunodeficiency virus–1 and hepatitis C virus) [31–33]. Selective transmission of a bloodborne virus after surgical exposure has not been reported. Surgeon-related outbreaks of HBV, which presumably occur as a result of percutaneous exposure, provide a unique opportunity to examine the phenomenon of selective transmission, since they have a clearly identifiable source, a precise time of exposure, and, often, multiple recipients associated with a single source.

In this study, we examined clones derived from the C gene of HBV in sera of HCWs in whom variation in HBV genetic heterogeneity would be expected, given that the HCWs were at varying stages of chronic HBV infection. We elected not to perform similar clonal analyses of sera from the infected patients. These individuals, in contrast to the HCWs, were undergoing acute HBV infection. At this stage of infection, virions shed into the circulation reflect vigorous shedding of the infecting HBV variant that is undergoing vegetative replication in the liver. Clonal analysis in the manner performed for the HCWs would be expected to yield identical sequences in nearly all clones. Consequently, the viral subgenomic sequence ob-

Figure 1. Electrophoretic autoradiographic banding patterns obtained by polymerase chain reaction (PCR)–single-strand conformation polymorphism (SSCP) assay of C gene fragment amplified from serum of transmitters and cases of representative clusters (C, D, F, G, and H) of hepatitis B virus (HBV) infection. P1, plasmid pHBV130; T, transmitter; and C, cases (all specimens taken at the time of acute infection). For D, SSCP bands for 2 of a total of 3 infected patients are shown (C1 and C2). For F, the top band of T is lower than C, and for G the lowest band of T is higher than C; these differences reflect single base mutations (see figure 2). For H, Ta and Tb designate specimens from transmitter taken 28 months apart; specimens of C1, C2, and C3 were taken 4, 2, and 2 months, respectively, before Ta.

Table 4 summarizes the distribution of PCR clone sequences of the 2 groups.

Figure 2. Alignment of C gene sequences between positions 1928 and 1984 and between 2068 and 2124, showing nucleotide differences between transmitter and case for each of clusters F, G, and H. Sequence of transmitter F is lettered. Dots signify identity to nucleotide of transmitter aligned above it. Bases in sequence of cases that differ from those of corresponding transmitter are underlined. GenBank accession numbers: transmitter F, AF204953; case F, AF204954; transmitter G, AF204955; case G, AF204956; transmitter H, AF204957; case H, AF204958.
Table 3. Comparison of characteristics of hepatitis B virus (HBV) derived from healthcare workers associated with nonselective and selective HBV transmission.

<table>
<thead>
<tr>
<th>Group</th>
<th>HBeAg status</th>
<th>Precore mutant present?</th>
<th>Circulating viral load (copies/mL)</th>
<th>Heterogeneity indexa</th>
<th>Heterogeneity of PCR clones derived from C gene (normalized Shannon entropy $S_n^b$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: transmission of dominant variant</td>
<td></td>
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<tr>
<td>A</td>
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<td>$10^9$</td>
<td>.2</td>
<td>.266</td>
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<td>B</td>
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<td>$10^5$</td>
<td>.352</td>
<td>.384</td>
</tr>
<tr>
<td>C</td>
<td>Positive</td>
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<td>$10^9$</td>
<td>.333</td>
<td>.427</td>
</tr>
<tr>
<td>D</td>
<td>Positive</td>
<td>No</td>
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<td>.328</td>
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<tr>
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<td>No</td>
<td>$10^9$</td>
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<td>II: transmission of minor variant</td>
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<tr>
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<td>$10^4$</td>
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<td>.816</td>
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<td>G</td>
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<td>$10^7$</td>
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<td>$10^5$</td>
<td>.667</td>
<td>.723</td>
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</table>

I vs. II (P) $1 > .05^c$ $1 > .05^c$ $1 > .05^c$ $>.0034^d$ $>.0087^d$

NOTE. HBeAg, hepatitis Be antigen; PCR, polymerase chain reaction.

Table 4. Distribution of sequences in polymerase chain reaction clones derived from hepatitis B virus C gene fragment of transmitter.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
<th>XI</th>
<th>XII</th>
<th>XIII</th>
<th>XIV</th>
<th>XV</th>
<th>XVI</th>
<th>Total</th>
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<tr>
<td>G</td>
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<tr>
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<td>15</td>
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</table>

NOTE. Roman numerals denote unique sequences. ST, selective transmission.
to possess genetic determinants that allow it, having entered into the liver of the new host, to establish an infection or replicate more readily than the variant that was dominant in the transmitter. The nonsynonymous base changes in the minority variant may either constitute the determinants themselves or cosegregate with determinants located elsewhere in the genome. Identification of such determinants might further the understanding of the widespread variation in the level of viremia among carriers, of the differences in clearance rates of HBV in vivo during antiviral therapy [34], and of the pathogenesis of fulminant hepatitis B, a condition associated with a high hepatic turnover of HBV [30, 35].

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References


