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NOTES

A Unique, Predominant Hepatitis C Virus Variant Found in an Infant Born to a Mother with Multiple Variants

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To demonstrate vertical transmission of hepatitis C virus (HCV) from an HCV-infected, non-human immunodeficiency virus type 1-infected mother to her infant and to assess the distribution of viral species in the mother and infant, the hypervariable region of the gene encoding the putative envelope glycoprotein E2 (E2HV) was sequenced in three mothers and one mother-infant pair. The data indicate that (i) quasispecies distributions of HCV E2HV variants were found in all four mothers, (ii) a single predominant HCV E2HV variant was found in the infant of a mother shown to have nine predominant E2HV variants, and (iii) the infant's E2HV variant was highly related to, but not identical with, the nine variants identified in the mother at the time of birth. These findings indicate that HCV is transmitted from mother to infant and raise the possibility that the transmission occurs in utero.

Hepatitis C virus (HCV) is a single-stranded RNA virus which is distantly related to the pestiviruses and flaviviruses and is the major agent responsible for posttransfusion and sporadic or community-acquired non-A, non-B hepatitis (reviewed in reference 9). Approximately 40% of non-A, non-B hepatitis cases are defined as community-acquired hepatitis, since the source of infection is not known (1, 2). Vertical transmission of HCV from mother to infant has been confirmed in mothers coinfected with human immunodeficiency virus type 1 (HIV-1) and HCV (6, 21), but transmission of HCV from a non-HIV-1-infected mother to her infant is strongly debated. Many studies have analyzed sera from infants 5 months of age or older (3, 10–12, 25) for the presence of HCV RNA and therefore cannot distinguish between vertical/perinatal and/or intrafamilial/horizontal transmission of HCV. Other studies, which include infants of less than 5 months of age, reported conflicting results regarding HCV RNA positivity in the infants evaluated (3, 12, 18, 25). To establish vertical transmission in HCV-positive, HIV-1 negative mothers and to assess the distribution of HCV variants in mother-infant pairs, we chose to sequence the most rapidly evolving region of the HCV genome (16, 17), the putative envelope glycoprotein 72 (gp72) hypervariable domain (E2HV) (8, 22), from a mother-infant pair. In the absence of a virus-neutralizing assay for HCV, the presence of virus-neutralizing epitopes in the E2HV domain has not been definitively established. However, the E2HV domain contains linear B-cell epitopes (23), and it has been hypothesized that the high degree of variability in this region may provide a mechanism by which the virus generates escape mutants and establishes chronic, persistent infections (23).

Results and discussion. Four of six C-100 Ortho ELISA (enzyme-linked immunosorbent assay)/RIBA II HCV antibody-positive mothers with a history of parenterally acquired HCV infection (four by transfusion and two by accidental needle stick) were found to harbor HCV RNA in their sera at the time of birth by cDNA-mediated polymerase chain reaction (cPCR) (19) using highly conserved primers from the 5′ terminal region of HCV (7). None of the mothers reported additional risk factors, and all tested negative for markers of HIV and HBV. When infant serum samples obtained 24 h after birth were analyzed by cPCR using primers from the 5′ terminal region and from the E2HV region (23) of the HCV genome, one of the six seropositive infants contained HCV RNA. The mother and infant were tested for anti-C25 (C25 = C100, C33C, and C22 combined into one molecule), anti-C22, and anti-C33C antibodies by a sensitive ELISA (4) at birth and 24 h after birth, respectively, and were shown to have identical levels of antibodies to all three antigens. (Use of these specimens was approved by the UCSF Institutional Review Board.)

Figure 1 shows the nucleotide (Fig. 1A) and deduced amino acid (Fig. 1B) sequences obtained from 10 individually M13 (14)-subcloned E2HV cPCR products from each of the four mothers (M1 to M4) and from the only HCV RNA-positive infant (B1). The data show that all four mothers had a quasispecies distribution (5) of E2HV nucleotide and amino acid sequences. A minimum of three E2HV variants (M2 and M3) and a maximum of nine E2HV variants (M1) were detected among the 10 clones evaluated in each group. In contrast, all 10 of the infant's sequences (B1.1 to B1.10) were identical to each other and were unique relative to the sequences of M1 to M4. A comparison of the B1 and M1 consensus amino acid sequence revealed that four of the six amino acid heterogeneities occurred between amino acids 395 and 407, which is the most rapidly evolving region of the E2HV domain (16, 17), and may be significant with respect to a biological function which has not yet been defined. The absence of nucleotide heterogeneity among the 10 B1 sequences and the high number of nucleotide changes

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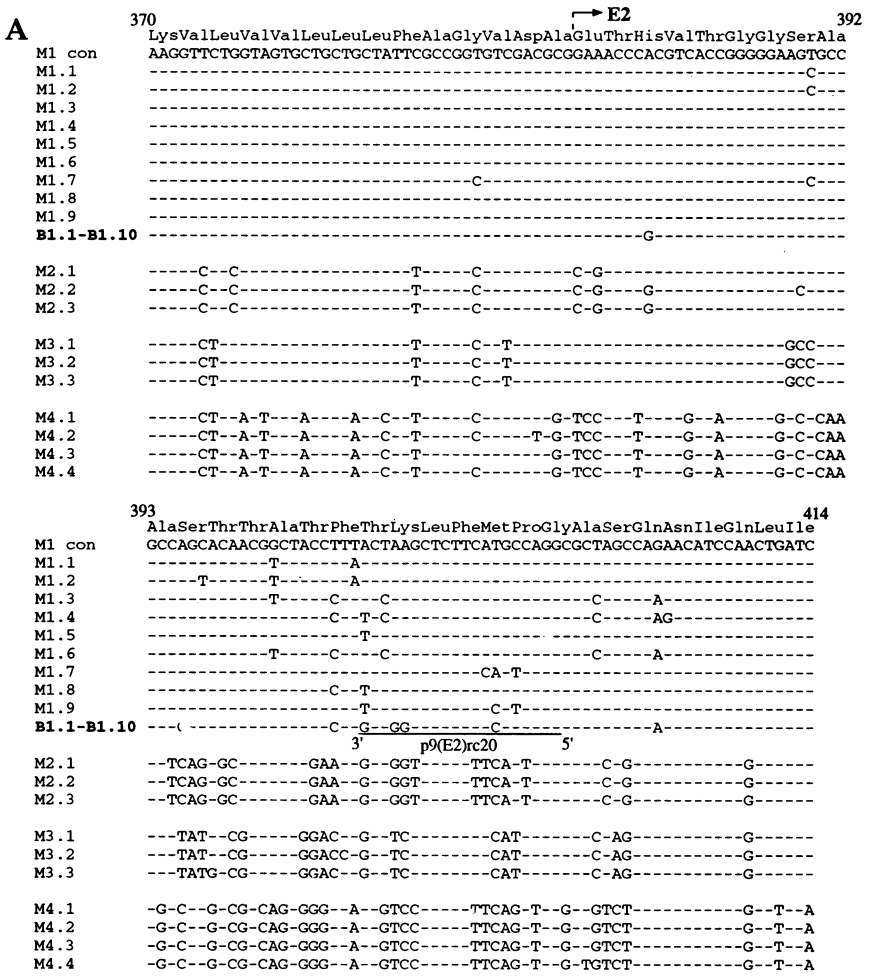

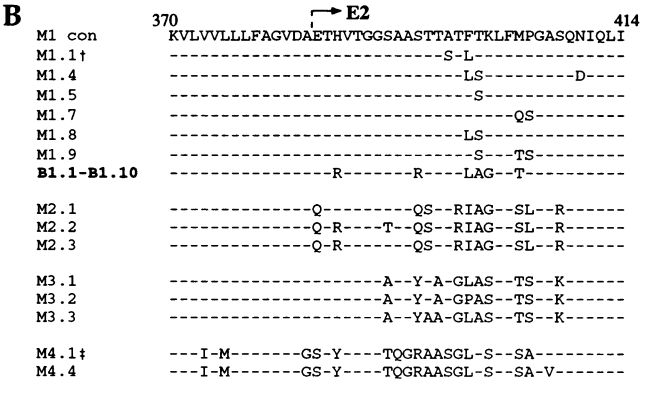

FIG. 1. (A) Unique nucleotide sequences of subcloned cPCR products from the HCV E2HV region of mothers M1 to M4 and infant B1; (B) deduced amino acid sequences for the corresponding nucleotide sequences in panel A. Dashes represent nucleotides or amino acids which are identical to the consensus sequence (con) given on the top line for each group of sequences. Unique sequences within each group are designated as M or B. 1-10. † or ‡ indicates that more than one nucleotide sequence (i.e., M1.1, M1.2, M1.3, M1.6, and M1.10 in panel A) has an identical deduced amino acid sequence (i.e., M1.1 in panel B). E2 designates the putative 5′ terminus of the gp72 HCV protein. The sequence of the infant-specific, antisense primer p9(E2)rc 20 is underlined.

in the 40 135-bp cPCR fragments derived from the mothers indicated that the heterogeneities observed among the E2 HV sequences are most likely authentic rather than due to misincorporation of nucleotides by either the reverse transcriptase or the *Taq* polymerase during transcription. The results extend the findings of Martell et al., who demonstrated that a single individual could have a population of HCV genomes containing point mutations in the 5′ terminal region and nonstructural protein 3 (NS3) gene (13) and indicates that a quasispecies distribution of E2HV variants is a general phenomenon in individuals with chronic HCV infections.

A phylogenetic tree (Fig. 2), constructed by pairwise, progressive alignment of the nucleotide sequences to one another (15) by using the computer software program GeneWorks Unweighted Pair Group Method with Arithmetic Mean, showed that the infant's unique HCV sequence (B1) was closely related to the population of variants from its own mother (M1) and was significantly divergent from the E2 HV variants from the three unrelated mothers (M2 to M4). Extensive effort was made to determine whether the B1 sequence could be detected in the RNA from the M1 serum obtained at birth. Using a B1-specific PCR primer [p9(E2)rc20 in Fig. 1A] in conjunction with primers X(E2)14, 18J, and 19J (21) in nested PCR and performing "hot start" PCR reactions (17a), which should prevent nonspecific priming at temperatures below the annealing temperature of the

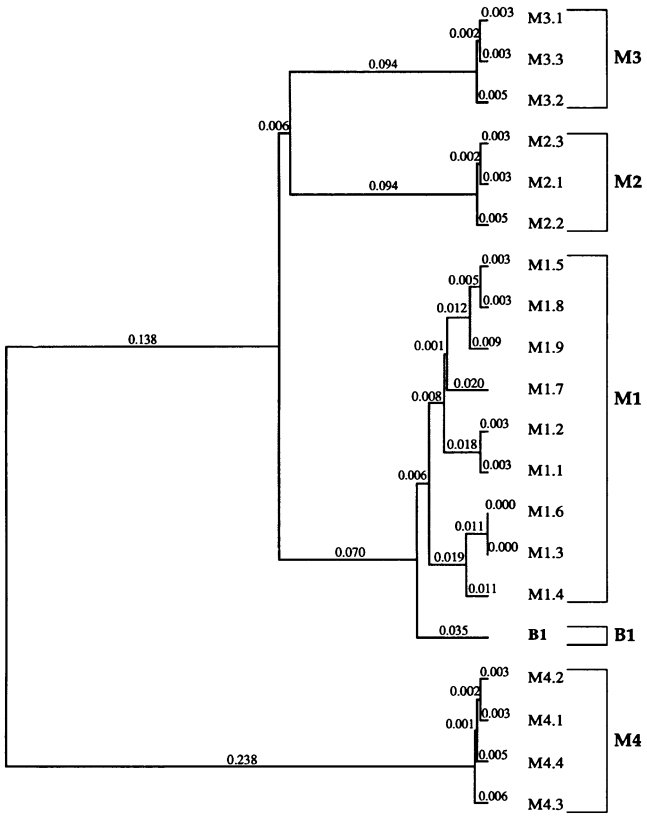

FIG. 2. Phylogenetic tree showing the genetic relatedness of each nucleotide sequence in Fig. 1A. The numerical values, derived according to reference 15, are proportional to sequence divergence.

primers under high-stringency reaction conditions (94°C denaturation for 10 s and 72°C annealing and extension for 60 s), we failed to identify the infant's sequence in the mother's serum RNA.

Since the B1 sequence was obtained from serum drawn from the infant 24 h after birth, which precluded external maternal blood contamination during the birthing process, the data provide unequivocal evidence that the mother, M1, transmitted HCV to her infant. Importantly, the predominant variant found in the infant differed significantly from all of the mother's nine predominant HCV sequences and could not be detected by cPCR in the mother. The inability to identify the infant's variant in the mother's serum was not likely due to poor sensitivity of the PCR primers, since we know from quantitative cPCR (24) that the infant and the mother both had easily detectable levels of HCV RNA (≥1,000 molecules per ml) and that the cPCR primers generated strongly staining ethidium bromide levels of PCR product when tested on RNA extracted from 40 μl of infant serum. The data argue against passive transfer of HCV from the mother to her infant since we did not find the maternal population of variants in the infant.

Although we cannot rule out the formal possibility that the transmission of the B1 variant was a result of a low-frequency, random event rather than a true selection process, our findings are very similar to those seen in HIV-1, in which case significantly less viral diversity was observed in the population of HIV-1 in three infants than in their infected mothers (26). It is possible that selection of variants of vertically transmitted viruses may be a general phenomenon which is not easily observed in viruses for which the degree of nucleotide substitution is not as high as it is in the V3 loop of HIV-1 gp120 or the E2HV domain of HCV.

Previous work indicates that HCV RNA is undetectable in experimentally infected chimpanzees, which have milder disease than humans, until 3 to 4 days postinoculation (20). If HCV replicates at a low level in human infants, our data raise the strong possibility that the infant may have been infected in utero by a virus whose sequence continued to diverge in the mother. In utero infection of the infant is also supported by the finding that only one unique, predominant variant was found in the infant. Alternatively, since we cannot absolutely rule out the possibility that the infant's sequence was present at levels which would make it undetectable in the mother by cPCR and since the kinetics of HCV replication in infants are unknown, the virus containing the unique B1 sequence may have had some selective advantage over other viral species in its ability to either cross the placenta or replicate efficiently in the new host. Taken together, these data suggest that HCV was transmitted from mother to infant either in utero or perinatally, and a selective process may have contributed to the lack of sequence diversity observed in the infant's HCV population. Understanding the pathogenesis of perinatally acquired HCV infection requires future long-term studies and may affect the clinical management of HCV-infected mothers and their infants.

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