Human Herpesviruses 6 and 7 in Cervixes of Pregnant Women

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We looked for human herpesvirus 6 (HHV-6) and HHV-7 genomes in the cervixes of pregnant women in the late stages of their pregnancies. Of 72 samples collected with cervical swabs and amplified by nested PCR, we found that 14 (19.4%) and 2 (2.7%) contained detectable HHV-6 and HHV-7 genomes, respectively. The two samples in which HHV-7 DNA was detected also contained HHV-6 genomes. Hybridization of HHV-6 DNA amplified by PCR with variant-specific probes revealed that all of these DNA samples belonged to variant type B. These results indicated that HHV-6 and/or HHV-7 exists in the cervixes of infected women in late pregnancy and may cause perinatal infection.

Human herpesvirus 6 (HHV-6), a new member of the human herpesviruses (11), is an etiologic agent of exanthem subitum, a common infant disease characterized by high fever and a cutaneous rash (17). The isolates of HHV-6 are classified into two distinct groups according to genetic, antigenic, and biological characteristics; the GS (or U1102)-like strain belongs to the variant A group and the Z29 (or HST)-like strain belongs to the variant B group (1). HHV-7 was identified for the first time in 1990 in CD4+ T cells from a healthy individual (5). Molecular, immunologic, and biologic studies demonstrated that HHV-7 is different from its closest relative among the human herpesviruses, HHV-6. HHV-7 was recently recognized as another causal agent of exanthem subitum (12). Serological studies show that most children have antibodies against HHV-6 and HHV-7 by the time they are 2 and 4 years old, respectively (10, 14). How these viruses are transmitted is not fully known. Several lines of evidence support horizontal transmission, possibly through oral secretions (7, 9). Vertical transmission has also been suggested as a mode of viral transmission, though this mode is not well understood (2–4, 13). In order to examine possible perinatal infection of HHV-6 and HHV-7 transmitted from mother to child, we evaluated cervical swabs from 72 pregnant women for the presence of DNA from HHV-6 and -7 by PCR.

The specimens were collected with cotton swabs from the cervixes of pregnant women 6 weeks prior to the date of delivery and from nonpregnant age-matched women. DNAs were extracted with Sepagene (Sanko Junyaku Co., Ltd.) according to the manufacturer’s instructions. Nested PCR for both HHV-6 and HHV-7 and hybridization with type-specific oligonucleotide probes were performed as described previously (15). Briefly, 10 μl of each sample was added to 40 μl of reaction mixture containing outer primers and Taq polymerase (Takara Shuzo Co.). For HHV-6 PCR analysis, the mixture was subjected to 30 amplification cycles of denaturing at 90°C for 1 min, annealing at 62°C for 2 min, and extension at 72°C for 3 min. Five microliters of the PCR product from the first round of amplification was used as the template for the second round of PCR. The conditions were the same as for the first PCR except that the inner primers were used instead of the outer primers. The sequences of the HHV-6 outer primers were 5′-TTTCCAGATGTGCCAGGAAATCC-3′ and 5′-CATCATTGTTATCGCTTTC-3′. The sequences of the inner primers were 5′-AGTGACAGATCTGGGCGGC-3′ and 5′-CACTAATAACTT-3′. The primers used for HHV-7 DNA detection were derived from the immediate-early gene loci of both variants A and B and HHV-7, which was isolated from a patient with exanthem subitum, and they were confirmed not to be able to amplify DNAs of HHV-6 and other human herpesviruses (unpublished data). A DNA sample extracted from U1102- or HST-infected cells was used as a positive control. Nested PCR fragments of HHV-6 and HHV-7 were detected by staining the gels with ethidium bromide. From sequence analyses the sizes of the products after nested PCR were expected to be 195 and 423 bp for variants A and B of HHV-6, respectively, and 264 bp for HHV-7. In order to confirm the results of PCR, hybridization with an alkaline phosphatase-conjugated oligonucleotide probe was performed. The sequences of the probes for HHV-6 variants A and B and HHV-7 were 5′-TAAATCCATTACTGGCCTTGAA-3′, 5′-AACTCCAATCAGCCGCCACTCAG-3′, and 5′-CATACTCCAGTGACCTTCCGAATT-3′, respectively.

Figure 1 shows the results of PCR. Of 72 samples, 14 (19.4%) (Fig. 1a) and 2 (2.7%) (Fig. 1b) contained detectable HHV-6 and HHV-7 genomes, respectively. The two samples in which HHV-7 genomes were found also contained HHV-6 genomes. None of the amplified DNAs were detected after a single round of PCR. In contrast to samples from the pregnant women, as few as 2 (6%) of 34 samples from nonpregnant controls contained HHV-6 genomes, and no HHV-7 genomes were found. The sensitivities for detection of the PCR products of the HHV-6 and HHV-7 genomes described above were 10²

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and 10^3 copies of DNA by single PCR and 10 and 10^2 copies by nested PCR, respectively (data not shown). These results indicated that the samples contained at least 10 copies of HHV-6 DNA and 10^2 copies of HHV-7 DNA.

As determined by electrophoresis, the sizes of all the PCR products corresponded to those of HST DNA but not to those of U1102 DNA (Fig. 1a). The PCR products could be classified by size, because the primers covered a region in variant B that was deleted in variant A (16). The results suggested that the HHV-6 genome types found in the samples were all variant B.

Dot blot hybridization with type-specific probes further confirmed the variant types. Figure 2 shows representative results of hybridizing type-specific probes with the samples that showed positive bands by PCR; all 14 samples were amplified by HHV-6 primers (in Fig. 2, results only for samples 1, 5, 9, 10, 12, 13, and 14 are presented), while 2 samples (no. 6 and 14) were amplified by HHV-7 primers. As expected, the 14 PCR products which hybridized to the variant B-specific probe did not bind to variant A- or HHV-7-specific probes. The two products amplified by HHV-7 primers hybridized only to probes specific for HHV-7. These results clearly confirmed the results shown in Fig. 1.

In this study we demonstrated the presence of HHV-6 and HHV-7 in the cervical swabs of pregnant women. The frequency of reactivation of HHV-7 was very low (2.7%), but that of HHV-6 was unexpectedly high (19.4%). We detected the genome of human cytomegalovirus in 22 (30.6%) of the same samples by a single round of PCR (data not shown). In contrast, neither HHV-6 nor HHV-7 DNA was detected by nested PCR in most of these cases. These findings indicated that the frequency of reactivation and the quantity of virus reactivated were lower for HHV-6 than for human cytomegalovirus.

The possibility of intrauterine transmission of HHV-6 from mothers to babies has been reported. Dunne and Demmler found HHV-6-specific immunoglobulin M antibody in a few umbilical cord blood samples, although no HHV-6 DNA was detected by PCR (4). Furthermore, Aubin et al. provided direct evidence of intrauterine HHV-6 transmission by analyzing tissues from a fetus by PCR (3). Wiersbitzky et al. documented cases of pre- and perinatal HHV-6 infection which can provoke dramatic clinical symptoms of illness resulting in death or cerebral defects (13). Some data suggest a relationship between HHV-6 infection and spontaneous abortion (2). In this study we also provided evidence of possible perinatal transmission during HHV-6 and/or HHV-7 infection. We tried to isolate HHV-6 by cultivating HHV-6 DNA-positive cervical samples from other pregnant women with cord blood mononuclear cells, but no virus could be isolated (unpublished data). However, this result does not exclude the possibility of the presence of infectious virus in cervixes, because virus isolation from samples in vitro is sometimes unsuccessful.

During preparation of this paper a similar report appeared (6). The authors investigated by PCR vaginal secretions of women attending a sexually transmitted disease clinic for the presence of HHV-6 DNA and observed that at least 10% of the specimens contained HHV-6 DNA. Further studies encompassing a larger patient base are necessary to understand the natural history of HHV-6 and HHV-7 and the clinical diseases caused by these viruses in infants.

REFERENCES