Transplacental Congenital Human Herpesvirus 6 Infection Caused by Maternal Chromosomally Integrated Virus

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Congenital human herpesvirus 6 (HHV-6) infection results from germline passage of chromosomally integrated HHV-6 (CI-HHV-6) and from transplacental passage of maternal HHV-6 infection. We aimed to determine whether CI-HHV-6 could replicate and cause transplacently acquired HHV-6 infection. HHV-6 DNA, variant type, and viral loads were determined with samples (cord blood, peripheral blood, saliva, urine, and hair) obtained from 6 infants with transplacentally acquired HHV-6 and with samples of their parents’ hair. No fathers but all mothers of infants with transplacentally acquired HHV-6 had CI-HHV-6, and the mother’s CI-HHV-6 variant was the same variant causing the transplacentally acquired congenital HHV-6 infection. This suggests the possibility that CI-HHV-6 replicates and may cause most, if not all, congenital HHV-6 infections.

Recently, we reported that 1% of newborns have congenital human herpesvirus 6 (HHV-6) infection and that most of these congenital infections (86%) resulted from chromosomally integrated HHV-6 (CI-HHV-6) [1, 2]. The remainder of the congenital infections were presumed to be due to maternal HHV-6 reactivation or reinfection with subsequent transplacental infection of the fetus, the recognized usual mode of congenital viral infections, as occurs with cytomegalovirus [3]. The integration of the viral genome into human chromosomes is a unique mode of congenital infection, and little information exists about the biological characteristics and clinical importance of the integrated virus. Unknown are whether the chromosomally integrated virus replicates and whether protective immunity to subsequent HHV-6 infection develops.

Infants with chromosomally integrated virus can produce antibody to HHV-6 [2], which may be in response to the active replication of HHV-6 from a postnatally acquired HHV-6 strain or from the chromosomally integrated virus. The biological implications of this are potentially important in the subsequent outcome and neurodevelopment of infants with CI-HHV-6 because the HHV-6 genome in those with CI-HHV-6 is present in all cell types, including those in the central nervous system [4, 5]. We therefore postulated that if the chromosomally integrated virus does replicate, women with CI-HHV-6 could have congenitally infected infants not only from germline passage of the chromosomally integrated virus, but also from transplacental passage of HHV-6.

Methods. We enrolled infants with congenital HHV-6 infection and their parents in our study on the mechanisms associated with transplacently acquired congenital infection with HHV-6 [1, 2, 6]. The families were approached for enrollment only after their private physicians approved of our contacting the family. The study was approved by the University of Rochester Subjects Review Board. Each family provided written informed consent.

Children with transplacentally acquired infection were those with HHV-6 DNA detected in cord blood samples but with viral loads that were lower (<1 genome-equivalent copy [GEC] per 10–102 leukocytes, equivalent to <1 GEC per microgram of cellular DNA [7–9]) than HHV-6 loads associated with CI-HHV-6 [8]. In addition, no child with transplacental HHV-6 infection had HHV-6 DNA in any hair follicle samples. Individuals with CI-HHV-6 were identified by having HHV-6 DNA detected in their hair follicle samples or by the consistent presence of high viral loads of HHV-6 DNA (≥1 GEC per leukocyte, or ≥1 × 105 to 2 × 103 HHV-6 GEC per microgram of cellular DNA), present in all blood samples [2, 4, 5]. The detection of HHV-6 DNA in hair follicle samples was used to identify CI-HHV-6 among the parents in this study.

Cord blood, peripheral blood, saliva, and urine samples were obtained from children up to 3 years of age and processed as described elsewhere [2]. The cord blood samples were obtained from free-flowing cord blood with procedures specifically designed to avoid maternal blood and other potential sources of...
contamination. Hair follicle specimens were also obtained from the children and their parents.

Nested polymerase chain reaction (PCR) assays involving virus-specific typing with oligonucleotide probes specific for HHV-6 variants A and B were conducted as reported elsewhere [2, 10]. The assays reliably detected \( \leq 10 \) GECs.

To detect replicating HHV-6, our reverse-transcription PCR (RT-PCR) assay amplifies the gp82–105 messenger RNA (mRNA) of HHV-6, as reported elsewhere [2]. This assay detected \(<10\) mRNA copies.

The quantitative PCR assay for the HHV-6 U38 gene was performed as described elsewhere [2]. Mean results from \( \geq 2 \) wells were reported as GECs of HHV-6 per microgram of cellular DNA.

HHV-6 antibody levels were determined by an indirect immunofluorescence serologic assay using a clinical HHV-6 isolate that contained HHV-6A and HHV-6B genomes. Positive results were defined as log2 titers of \( >3.32 \) (\( >1:10 \) dilution).

Results. We identified 6 children with transplacentally acquired congenital HHV-6 infection. Three of these children were infected with variant HHV-6A, and the other 3 were infected with HHV-6B (Table 1). The HHV-6 levels detected in their cord blood ranged from 2.35 to 4.18 log10 GEC per microgram of cellular DNA, which is characteristic of transplacentally acquired infection and below that observed with infections resulting from CI-HHV-6 (\( \geq 5.1 \) log10 GEC per microgram of cellular DNA) [2]. In the subsequent 5 peripheral blood samples obtained from the children between 6 and 25 months of age, HHV-6 DNA was detectable in only 2 samples and at similarly lower levels (Table 1). Of the saliva samples that were collected from the children from 2 to 25 months of age, HHV-6 DNA was identified in 9 (47%) of 19 samples, but the HHV-6 DNA was not consistently detected among any child’s samples. No HHV-6 DNA was detected in any of the urine samples. Active replication, as detected by the presence of HHV-6 mRNA, was not demonstrated in any of the HHV-6 DNA–positive cord blood, peripheral blood, or saliva samples.

Hair follicle samples obtained from the children and from each set of parents were examined for the presence of HHV-6 DNA by PCR. None of the hair follicle samples from the children contained HHV-6 DNA, thus confirming that CI-HHV-6 was not the cause of the congenital infection. None of the hair follicle samples from the fathers contained HHV-6 DNA, but all of the hair follicle samples from the mothers had HHV-6 DNA, indicating that all of the mothers had CI-HHV-6 (Table 1). The rate of CI-HHV-6 (6 of 6) among these mothers of infants with transplacentally acquired congenital infection was significantly greater than the expected rate of CI-HHV-6 among the general population, 1 of 116 (\( P<.0001; \) Fisher exact test) [2].

In further support of the presence of CI-HHV-6 in each mother, the viral loads in the maternal hair samples were all \( \geq(1-2) \times 10^3 \) HHV-6 GEC per microgram of cellular DNA, and the variant of the HHV-6 DNA detected in the maternal hair follicle in all cases was the same as that detected in the infant’s cord blood and in postnatal specimens. One child whose mother had variant A also had HHV-6A in his cord blood and initial saliva sample at 10 weeks of age. However, his subsequent saliva samples obtained after he was 1 year of age contained variant B, indicating postnatal acquisition of primary HHV-6B infection.

Discussion. We have previously shown that mothers with
chomosomally integrated HHV-6 may give birth to infants with congenital HHV-6 infection acquired by 2 different mechanisms, germline passage of chromosomally integrated HHV-6 or by transmission of maternal HHV-6 infection across the placenta [2, 11]. However, the findings from the families in this study, although limited, suggest that most, if not all, transplacentally acquired infections are associated with the presence of chromosomally integrated virus in the mother.

One explanation for this finding is that the HHV-6 DNA detected in these infants results from the transplacental passage of virus from the maternal chromosomally integrated HHV-6, which is biologically active. Several other explanations should be considered. Transplacentally passed HHV-6 could result from maternal reinfection with a new strain of HHV-6. Alternatively, maternal peripheral blood mononuclear cells containing the integrated virus could be transferred through the placenta from mother to child [12]. Against this possibility, however, is that the HHV-6 DNA was detected in half of the saliva samples obtained from these children; because none were breast-fed, the source of the detected HHV-6 DNA could not be breast milk. Contamination of the cord blood sample at the time of delivery with maternal blood is also possible. However, our procedures for collection of cord blood samples were specifically designed to avoid contamination with maternal blood.

More importantly, we have identified the presence of HHV-7 DNA in the peripheral blood of 67% of pregnant women, yet we have not detected HHV-7 DNA in any of the more than 2000 cord blood samples examined [1, 11].

Transplacental passage of the chromosomally integrated strain is supported by our findings that the HHV-6 DNA variant detected in the mothers was the same as that in their infants, and that it was HHV-6 variant A in one-half of these cases. HHV-6 variant A is detected significantly more frequently among those with CI-HHV-6 than among the general population of children with postnatally acquired infection [1, 2]. Variant A accounts for only 1%–3% of the HHV-6 detected in the peripheral blood mononuclear cell and saliva specimens of children and their families [13]. Larger studies of children with transplacentally acquired congenital HHV-6 infection and their families are needed to confirm and expand these observations. Such studies, however, are confounded by the low prevalence of congenital HHV-6 infection resulting from transplacentally acquired virus. Although ~1% of individuals have congenital HHV-6 infection [1], only ~14% of them, or ~1 of every 714 newborns, have transplacentally acquired infection [2]. The neurodevelopmental outcome of children with congenital infection from HHV-6 is currently unknown. However, confirmation of these findings would suggest that all congenital HHV-6 infections could be feasibly detected prenatally by determination of HHV-6 DNA in parental hair samples.

References