Cord blood DNA was tested for the presence of human herpesvirus 6 (HHV-6) DNA by the polymerase chain reaction. Specific DNA could be detected in the specimens of 5 (1.6%) of 305 babies born to ostensibly healthy mothers, indicating that intrauterine infection had occurred. These transmissions would not have been detected by serologic methods, because no specific IgM antibody could be found in the fetal sera. These results indicate that, in addition to infections acquired in early childhood, congenital infections may account for the HHV-6 seropositivity in children.

Primary infection with human herpesvirus 6 (HHV-6) may result in exanthem subitum (roseola infantum), a common febrile illness with rash in infancy [1]. It has been implicated as a cause of encephalitis and recurrent seizures [2]. Its clinical significance in immunocompromised patients after bone marrow transplantation is under active consideration [3].

Because HHV-6 DNA has been detected in saliva of ≥90% of healthy persons by the polymerase chain reaction (PCR) [4], transmission via oral secretions is thought to be the major route of infection. By their first birthday, >70% of children have experienced infection with HHV-6 as judged by the prevalence of specific antibody [5]. Hitherto, the youngest infants with virologically confirmed HHV-6 infection were ~3 weeks old [6]. Since HHV-6 DNA has been detected in cervical swabs of pregnant women [7], the possibility of perinatal infection has been suggested.

Thus far, intrauterine infection has not been demonstrated as a mode of HHV-6 transmission. There are isolated reports of HHV-6 detection in fetuses following spontaneous abortions [8] or interruptions [9]. Serologic studies for HHV-6-specific antibodies have revealed contradictory results [5, 10]. Previous attempts to demonstrate the presence of HHV-6 in cord blood have been unsuccessful [10, 11].

The present report demonstrates that intrauterine transmission of HHV-6 can occur as shown by DNA PCR in cord blood specimens.

Materials and Methods

**DNA samples.** Between November 1994 and June 1995, 305 cord blood specimens were obtained from the Obstetrics Department and stored in the Cord Blood Bank of the Bone Marrow Donor Center of Heinrich Heine University. The method for collection and processing of cord blood has been described in detail [12]. Maternal venous blood specimens were taken immediately after delivery. Maternal sera were tested for antibodies against hepatitis B and C viruses, human immunodeficiency virus (HIV) types 1 and 2, human T lymphotropic virus types I and II, cytomegalovirus, and Epstein-Barr virus. DNA was extracted from cord blood and maternal white blood cells after gradient separation as described [12]. Maternal cell contamination of the cord blood was excluded by the negative outcome of PCR amplifications of the noninherited HLA-DRβ exon 2 [12]. DNA derived from 1.5 × 10^6 peripheral blood lymphocytes (PBL) was used in each PCR reaction.

**PCR analysis.** Two sets of PCR primers and probes were used for the detection of HHV-6 DNA. One set of HHV-6–specific primers, designated pr598 and pr599, and a biotinylated probe derived from a region near the left end of the “portion unique” region between map positions 4063 and 4454 [13]. Positive results were confirmed using primers specific for a different part of the HHV-6 genome (i.e., the major capsid protein) [14]. This reaction was done with primers HHV-103 (5’-CAATGCCTTCTAGCCGCCTCTTC) and HHV-104 (5’-ACATCTATAATTTAGACGATCC) and the biotin-labeled probe (5’-TGATGTCCGATCCACGTATTATGTCCCGTAAAC) (Genemed Biotechnologies, San Francisco). The map position of the resulting 479-nt PCR product corresponds to nt 4834–5313 of the 4L region [14].

PCR reactions were performed under standard conditions for 35 cycles (15 s at 95°C, 15 s at 60°C, 1 min at 72°C) using a thermocycler (model 9600; Perkin-Elmer, Norwalk, CT). After electrophoresis on agarose gels, the PCR products were blotted onto a membrane and hybridized with the biotin-labeled probe, followed by incubation with a streptavidin-linked alkaline phosphatase and nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate. DNA extracted from JJHAN cells infected with a high-replicating wild-type HHV-6 isolate served as a positive control. Limiting dilution of HHV-6 infected cells in uninfected JJHAN-cells was done to determine the sensitivity of the PCR. A positive signal could be
The reaction of CB144 was strong in the first PCR and weaker in the second; all others showed strong reactions in the PCR with both sets of primers.

HHV-6 DNA could not be detected in any of the DNA specimens of the corresponding mothers. β-actin PCR of all samples used in this experiment showed no differences between fetal and maternal DNA preparations (figure 1C), ruling out the possibility that the failure to detect HHV-6 DNA in maternal specimen was due to the presence of PCR inhibitors. Thus, the positive results in cord blood DNA could not be due to contamination with maternal material.

The failure to detect HHV-6 DNA in maternal specimens or, generally speaking, in latently infected individuals is due to the low sensitivity of the single-round PCR used in the present study. Other investigators could regularly find HHV-6 DNA in healthy adults only when they used $1 \times 10^4$ PBL per reaction and a $^{32}P$-labeled probe [4]. Another group [6], using nested PCR, detected HHV-6 DNA in $60\%$ of samples obtained 2 months to 2 years after primary infection.

The sequences of DNA amplified in the first PCR of the 5 cord blood DNAs and of the DNA of our laboratory isolate and differed from the published sequence in 4 silent mutations in the coding positions of the HHV-6 major capsid protein (codon 13, GTC[V] to GTT[V]; codon 29, CTT[L] to CTG[L]; codon 32, CAT[H] to CAC[H]; and codon 61, TTT[F] to TTC[F]) and in 1 position in the noncoding region (G to A at nt 21 upstream of the start codon). The other 4 amplified sequences were identical to the published sequence of U1102 [13]. High degrees of conservation of DNA sequences are common in herpes viruses.

Since all positive results could be confirmed with a second DNA sample and a second set of PCR primers (see Materials and Methods), they cannot be due to contamination. Other sources of HHV-6 DNA can be excluded, because we have not worked with HHV-6 DNA in any context beyond that described here, and the U1102 DNA purposely has not been introduced into this laboratory.

Serologic testing of the sera of the 5 children and their mothers revealed the presence of IgG antibodies to HHV-6 in all cases (table 1) but no IgM antibodies. The quantitative IgG analysis showed, in 4 cases, lower levels in cord blood than in the maternal serum. Retroactively, it would have been desirable to demonstrate the continued seropositivity to HHV-6 in the babies with demonstrable HHV-6 DNA; however follow-up of the newborns has not been scheduled in the present protocol.

**Discussion**

This is the first report describing the detection of HHV-6 DNA in cord blood cells of babies born to healthy mothers.
Table 1. Titters of HHV-6 specific antibodies in cord blood and corresponding maternal sera.

<table>
<thead>
<tr>
<th>Pair no.</th>
<th>Source</th>
<th>IgG</th>
<th>IgM</th>
</tr>
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<tbody>
<tr>
<td>131</td>
<td>Mother</td>
<td>320</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>Cord blood</td>
<td>320</td>
<td>&lt;20</td>
</tr>
<tr>
<td>144</td>
<td>Mother</td>
<td>640</td>
<td>&lt;20</td>
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<tr>
<td></td>
<td>Cord blood</td>
<td>160</td>
<td>&lt;20</td>
</tr>
<tr>
<td>208</td>
<td>Mother</td>
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<td>&lt;20</td>
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<td>&lt;20</td>
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<tr>
<td>320</td>
<td>Mother</td>
<td>5120</td>
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</tr>
<tr>
<td></td>
<td>Cord blood</td>
<td>320</td>
<td>&lt;20</td>
</tr>
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</table>

In the context of using cord blood–derived stem cells for allotransplantation, the present findings warrant further study of the pathogenic potential of HHV-6 in immunocompromised patients in order to more accurately determine the need for exclusion of HHV-6–positive cord blood from clinical applications.

It has been suggested that infection or reactivation of HHV-6 during the early stages of pregnancy may predispose to spontaneous abortion [8]. However, at present there are no clear indications that intrauterine transmission of HHV-6 has any ill effects on the newborn.

Acknowledgments

We thank K. E. Schneweis for providing the JJHAN cells and the HHV-6 isolate and S. Scherger for excellent technical assistance.

References

10. Dunne WM, Demmler GJ. Serologic evidence for congenital HHV-6 transmissions. In a study involving 799 newborns, 2 cord blood specimens contained demonstrable IgM antibodies to HHV-6, but no genomic HHV-6 DNA could be found in those 2 specimens [10]. In another study [5], HHV-6 IgM antibodies could not be found in 235 cord blood samples. Both studies used IFA for detection of antibody. In earlier work utilizing IFA with cytomegalovirus [15], it had been shown that only 45%–80% of babies congenitally infected with cytomegalovirus could be identified by detection of specific IgM. These results justify concerns about the use of IFA for the diagnosis of congenital infections with herpesviruses. These concerns are further emphasized by the fact that in no case of the 5 congenital infections described in the present study could IgM or elevated IgG levels be found.

In one report, HHV-6 DNA has been detected in a fetus of a mother with HIV-1 infection [9], suggesting reactivation of HHV-6 and intrauterine transmission in a case of compromised maternal immunity. In the present study, all mothers were ostensibly healthy and negative for HIV-1 antibodies.