NOTES

Viral Load in Breast Milk Correlates with Transmission of Human Cytomegalovirus to Preterm Neonates, but Lactoferrin Concentrations Do Not

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Breast-feeding is a strong risk factor for the postnatal transmission of human cytomegalovirus (HCMV) (2, 21). The rate of transmission by consuming HCMV-infected breast milk ranges from 58 to 76% (3, 24).

Although HCMV-infected cells have been isolated from breast milk (1, 5) and cell-free virus has been detected in the whey of HCMV-infected mothers (1, 5), the mechanism of virus transmission through breast milk has not been elucidated yet. In contrast, HCMV is seldomly detected in colostrum (16). Breast milk has a protective effect against microbial infections and one of the protective components is lactoferrin (LF). LF, an 80-kDa iron-binding glycoprotein, is present in the secondary vesicles of neutrophilic granulocytes (12). LF is also present in mucosal secretions (11, 13), where it is produced by epithelial cells, e.g., by the mammary glands during lactation (11, 13). At the mucosa, LF exerts its antibacterial and fungicidal effect (10, 11, 13). In vitro, LF exerts antiviral activities against a plethora of viruses, including hanta, HIV and HCMV (6, 15, 18, 22).

Lactoferrin concentrations are highest in colostrum and tend to decrease significantly within the first weeks of lactation (7, 14). We hypothesized that LF, among other defense proteins, would help to prevent the transmission HCMV to the newborn. In particular, for preterm newborns this nonspecific immunological defense could be important.

We set out to determine the LF concentrations in breast milk longitudinally to assess the relation between transmission of HCMV and LF levels in vivo. The relation between LF concentrations and the total amount of HCMV DNA in breast milk was studied in the same samples.

Study group. Breast milk specimens were obtained from 23 breast-feeding mothers of preterm infants at the University Hospital of Tübingen. These mothers were enrolled prospectively between July 1995 and June 1998 in a clinical study of postnatal mother-to-preterm infant transmission of HCMV via breast milk (4). HCMV screening of seronegative and seropositive mother-infant pairs was performed by serology, virus culture, and PCR. Congenital and perinatal HCMV transmission were excluded. All mothers were informed of the aim of the study, which was approved by the ethical committee of the University of Tübingen. All mothers were without clinical symptoms of HCMV infection and were classified into four groups. The first group were seronegative controls (group 1, n = 4), i.e., without transmission, DNA-lactia, and virolactia. Groups 2 (n = 4), 3 (n = 8), and 4 (n = 7) all comprised seropositive mothers with DNA-lactia. Transmission only occurred in group 4, for which the mothers, as in group 3, had virolactia. Group 2 mothers had no virolactia.

Milk whey preparation. Native expressed breast milk was sampled longitudinally. Cell-free milk whey was prepared as described previously (5) and stored as aliquots at −20°C.

DNA extraction and qualitative nPCR from milk whey. The extraction of DNA and detection of HCMV DNA by nested PCR (nPCR) in milk whey was performed as previously de-
scribed (5). This approach allowed detection of 200 genome equivalents (GE) per ml of milk whey.

**Determination of viral load by quantitative nPCR.** Extracted DNA from breast milk samples were added to PCR reaction mixtures containing 50 copies (high standard) or 10 copies (low standard) of a cloned CMV standard (9, 17). Target sequences were amplified with the external CMV-specific primers E1 and E2 (17). Then, 5 μl each of the external reaction was reamplified in a second round of PCR with the internal CMV-specific primers TGGE1B and TGGE2E. Standard and wild-type CMV PCR amplimers were quantitated by hybridization analysis as described elsewhere (17). For CMV DNA copies of ≥20 in 2.5 μl, the data from the high-standard reaction were used, and for CMV copies of <20, data from the low-standard reaction were used. Results were expressed as the number of CMV wild-type GE per ml of milk whey. Exact quantification was possible at between 400 to 200,000 GE/ml.

**Detection of virolactia and transmission.** HCMV was cultured from milk whey by using human foreskin fibroblasts in the tube cell culture system. Virus transmission to the preterm infant was documented by positive viruria or DNA-uria not earlier than 3 weeks after delivery. Viruria was detected by

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**FIG. 1.** LF concentrations in breast milk decrease during lactation. Using smoothing spline fitting, no significant differences in decline or initial altitude of LF levels in mature milk were observed.
that the transmission of cell-bound virus in vitro could only be inhibited for 50% (8).

The reason for the more variable breast milk LF concentrations in the transmitter group could be reflected by different degrees of local inflammation in the breast (13). It is conceivable that, when large amounts of virus are present in breast milk, there also is viral replication in the breast, leading to a local inflammation reaction. As a result of this inflammation, the viral load in the transmission group (group 4) could have increased above a threshold level, which would lead to transmission and primary infection of the newborn. Although in vitro and in vivo data show that HCMV can replicate in several cell types (19, 20), the exact replication site in the mammary gland is not known.

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