Vol. 43, No. 3

Human Cytomegalovirus Reactivation during Lactation and Mother-to-Child Transmission in Preterm Infants

Johannes Meier,[1] Uta Lienicke,[2] Edda Tschirch,[2] Detlev H. Krüger,[1]
Roland R. Wauer,[2] and Susanna Prösch[1]*

*Institute of Virology[1] and Department of Neonatology,[2] Charité–University Medical School Berlin,
Humboldt University Berlin, Berlin, Germany*

In a clinical trial, the incidence of cytomegalovirus reactivation in breastfeeding mothers and transmission to their preterm infants were studied. Breast milk from 73 mothers as well as urine and tracheal and pharyngeal aspirates from their 89 infants were screened weekly for human cytomegalovirus (HCMV) DNA during the first 2 months after delivery. Of the 73 mothers, 48 (66%) were positive for HCMV DNA in the lactating breast. HCMV reactivation could be confirmed for 19 of 20 (95%) immunoglobulin G-positive mothers. Of the eight immunoglobulin G-negative mothers one was positive for HCMV DNA in breast milk. In only 2 out of 13 seropositive mothers with HCMV DNA in breast milk could viral DNA be detected in the peripheral blood. HCMV mother-to-child transmission was concluded for 20 of the 48 (42%) mothers positive for DNA or 7 of 19 (37%) seropositive for HCMV and positive for HCMV DNA in breast milk and one of one mother seronegative for HCMV but positive for HCMV DNA in breast milk. One mother transmitted the virus to her twins. In addition, one infant acquired postnatal HCMV infection despite the mother's being negative for HCMV DNA in breast milk; altogether, we found 22 infants with HCMV infection. In 13 of these 22 infants, virus infection occurred definitively postnatally; two of them developed severe symptomatic HCMV infection. HCMV-infected infants demonstrated higher incidences of amniotic infection, respiratory distress syndrome, bronchopulmonary dysplasia, and retinopathia praenatalis than noninfected infants, however, the differences were not statistically significant. In summary, our study confirmed a very high incidence of HCMV reactivation in mothers during lactation and a significant risk of transmission to preterm infants with the possibility of severe disease in these babies.

Human cytomegalovirus (HCMV) is the most common congenital infection. About 1 to 3% of newborns become infected during pregnancy. Around 10 to 15% of the infants born after maternal seroconversion are symptomatic, and less than 3% after reactivation (29). The main reason for prenatal HCMV transmission is primary infection of mothers during pregnancy rather than recurrent infection. Of even higher epidemiological relevance, however, is postnatal mother-to-child transmission, with breastfeeding being the main reason. In different studies, virolactia, or viral DNA in breast milk, was detected in 13 to 70% of lactating mothers (recently reviewed in references 6 and 32). Using highly sensitive methods like PCR to screen for viral DNA in breast milk or cell-free milk whey, it has been demonstrated that 40% to 96% of seropositive mothers shed the virus via their breast milk (2, 11, 14, 20, 30, 32).

The reason for DNA and viruses in breast milk was recurrent HCMV infections of the mothers during lactation. Interestingly, reactivation of HCMV has been mostly found restricted to the lactating breast, where the site of latency and virus production as well as the mode of reactivation are still unclear. Most of the virus in the breast milk was found to be free rather than cell associated and HCMV shedding followed defined kinetics with peak levels around 1 month after delivery (2, 11, 30, 32). To date the only confirmed sites of HCMV

latency in humans are undifferentiated progenitor cells of monocytes/granulocytes/dendritic cells in the bone marrow (10, 15, 19, 27). Systemic inflammation and stress are the two known mechanisms triggering HCMV reactivation from latency (7, 9, 24, 25).

In different studies postnatal transmission rates of HCMV to term and preterm infants varied between 10% and 60% (2, 3, 8, 11, 20, 28, 32). In the majority of postnatally infected children the HCMV infection remained clinically asymptomatic, however, preterm infants with very low birth weight were found to be at high risk to develop severe HCMV disease. Authors of different studies reported HCMV-associated disease in 0 to 87% of postnatally infected preterm infants (2, 3, 11, 16, 20, 23, 30-32). In our recent study on the role of HCMV for development of bronchopulmonary dysplasia we observed an increased incidence of this severe lung disease in HCMV-infected in comparison to noninfected infants. However, the difference was not statistically significant (23). In this bronchopulmonary dysplasia study we found severe HCMV disease in 1 of 16 (6%) infected preterm infants.

To determine the incidence of HCMV shedding in breast milk among mothers of preterm infants and HCMV infections in their preterm infants, we screened 73 mothers and their 89 preterm infants for HCMV DNA in cell-free milk whey and urine and tracheal-pharyngeal aspirates, respectively. The frequency of virus reactivation during lactation and transmission to the infants was determined for 28 mothers for which the HCMV serostatus was known. Our results strongly support that HCMV is reactivated in nearly all latently infected moth-

* Corresponding author. Mailing address: Humboldt University, Medical School (Charité), Institute of Virology, Schumannstr. 20/21, D-10117 Berlin, Germany. Phone: 49-30-450-525-121. Fax: 49-30-450-525-092. E-mail: susanna.proesch@charite.de.

TABLE 1. Clinical characteristics of uninfected and HCMV-infected infants[a]

Parameter	Total ($n = 89$)	Uninfected ($n = 67$) (75%)	HCMV infected ($n = 22$) (25%)	P
Gestational age, wk (range)	28 (24–33)	29 (24–33)	8 (24–32)	0.75
Birth weight, g (range)	1,119 (380–2,010)	1,133 (390–1,825)	1,048 (380–2,010)	0.3
Gender (male/female)	54/35	42/25	12/10	0.5
Delivery (vaginal/caesarean)	26/63	20/47	6/16	0.72
Amniotic infection (%)	43 (48)	33 (49)	10 (45)	1.0
Neonatal infection (%)	37 (41)	26 (39)	11 (50)	0.14
Assisted respiration, days (range)	10 (0–83)	9 (0–46)	14 (0–83)	0.39
Retinopathia neonatorom	19 (22)	12 (18)	7 (32)	0.19
Respiratory distress syndrome	44 (49)	30 (45)	14 (64)	0.13
Bronchopulmonary disease				
BPD 1	31 (35)	22 (33)	9 (41)	0.5
BPD 2	12 (13.5)	7 (10)	5 (23)	0.17

[a] Data are expressed as median and range. Differences between the CMV group and the no-CMV group are not statistically significant ($P > 0.05$). Amniotic infection was defined as a maternal CRP of >2 mg/dl and body temperature of $\geq 38°C$; neonatal infection was defined as an infant CRP of >1.0 mg/dl within the first three days of life or IL-6 level of >100 pg/ml. BPD, bronchopulmonary dysplasia with BPD_1 defined as an oxygen requirement of >25% on 28th day of life and BPD_2 defined as oxygen requirement at 36 weeks of postconceptional age.

ers and is shed within the breast milk. Preterm infants were found to be at significant risk of acquiring asymptomatic HCMV infection and even severe HCMV-associated disease. Moreover, HCMV infection in preterm infants may contribute to the development of chronic lung diseases.

MATERIALS AND METHODS

Patients and study design. In a prospective clinical trial between May 1999 and May 2003, 73 breastfeeding mothers and their 89 preterm infants (including 14 twins and one triplet) admitted to the University Hospital Charité were screened for HCMV in a weekly time course over the first 1 or 2 months after delivery.

Birth weights ranged from 380 to 2,010 g (median, 1,119 g), the median gestational age was 28 weeks (range, 24 to 33 weeks) (Table 1). Infants with prenatal HCMV infection as well as serious malformations and undetermined metabolic disorders were excluded from the study, as were infants who died within the study period. For blood transfusions only HCMV-seronegative blood products from the hospital's blood bank were used. Infants were fed fresh untreated breast milk from their own mothers eight times daily via a nasogastric tube. Feeding was started as early as possible, normally between 24 and 48 h after birth, with 1-ml portions that were increased daily by 1 to 2 ml/kg/portion up to one-sixth of their body weight. If maternal milk production was delayed, a special formula for preterm infants was given at the same frequency and dose until sufficient breast milk was produced by the mother. These studies were approved by the Ethics Committee of the Medical School Charité, and informed consent was obtained from all mothers. Sixty-one of the 89 infants had been enrolled in the study on HCMV infection and bronchopulmonary dysplasia as published very recently (23).

Specimens. Breast milk was collected weekly starting at day 3 postdelivery. For HCMV antibody testing, peripheral blood from 28 mothers was taken and tested for HCMV immunoglobulin M (IgM) and IgG during the first week postpartum. Additionally, from 19 of these 28 mothers citrated blood was collected during the first week after delivery for isolation of peripheral blood mononuclear cells (PBMC). Preparation of PBMC was performed immediately after collection by standard Ficoll/Paque gradient centrifugation (25). PBMC were stored at $-20°C$ until DNA preparation.

To determine the HCMV transmission rate tracheal aspirates from ventilated babies and pharyngeal aspirates from spontaneously breathing infants as well as urine samples were collected weekly and immediately frozen at $-20°C$ until DNA preparation as described in more detail elsewhere (23). Tracheal aspirates were collected after installation of 0.5 ml of sterile saline into the endotracheal tube by gentle aspiration through the endotracheal tube in a sterile tube.

Nucleic acid preparation and PCR. Isolation of DNA from tracheal aspirates and pharyngeal aspirates and urine and HCMV PCR were done as described recently (23). Breast milk was repeatedly centrifuged to eliminate fat globules and milk cells to generate cell-free milk whey (12). Milk whey was then passed through a sterile filter (0.2 μm; Schleicher & Schüll, Dassel, Germany) aliquoted, and stored at $-80°C$ until performing PCR. Citrated blood was separated on Ficoll/Paque gradients to isolate PBMC (23). DNA from milk whey, PBMC, and urine as well as tracheal aspirates and pharyngeal aspirates was isolated with the QIAamp blood Kit (Qiagen, Hilden, Germany) or the Pure Viral Nucleo Acid kit (Roche, Mannheim, Germany) after confirming comparable efficiency in DNA isolation of both kits. Routinely, DNA was isolated from 200 μl of urine, tracheal aspirates and pharyngeal aspirates, or cell-free milk whey and finally resolved in 50 μl of buffer as recommended by the manufacturer.

HCMV DNA amplification was carried out in a 50-μl reaction volume containing 10 μl of DNA with a one-step PCR and 40 cycles of amplification with 30 s of denaturation at 92°C, 1 min of primer annealing at 55°C, and 1 min of primer extension at 72°C. The sensitivity of the PCR was determined to be ≤ 50 copies of viral DNA per reaction. For amplification, HCMV-specific DNA primers complementary to the major immediate-early (IE) region were used. These primers amplified a 123-bp fragment of the IE1 protein coding sequence as described earlier (21-23). The PCR products were separated by 3% agarose gel electrophoresis and stained with ethidium bromide. The specificity of the amplified DNA fragments was confirmed by Southern hybridization with the internal oligonucleotide probe P2 terminally labeled with [γ-^{32}P]ATP (Hartmann Analytik, Braunschweig, Germany) and T4 polynucleotide kinase (Roche, Mannheim, Germany) (21-23). DNA samples negative for HCMV were probed for successful DNA preparation by beta-globin PCR according to Bauer et al. (3).

Serology. Serum IgM and IgG anti-HCMV antibody concentrations were determined by enzyme-linked immunosorbent assay techniques with the CMV-IgM enzyme-linked assay (Medac, Hamburg, Germany) and ETI-Cytok-G Plus (Sorin Biomedica, Saluggia, Italy), respectively.

Procedure. Breast milk, tracheal aspirates and pharyngeal aspirates, and urine specimens were collected at days 3, 7, 14, 28, 35, 42, 49, 56, and 64 postpartum and frozen until DNA preparation and PCR analysis. PBMC were prepared immediately after collecting the citrated blood. DNA preparation and PCR of specimens from the same individual were done independently on different days and in different PCRs to minimize the risk of false-positive results by contamination. Active HCMV infection was defined by repeated amplification of viral DNA from at least three consecutive urine and/or tracheal aspirate and pharyngeal aspirate samples.

Statistical analysis. Statistical significance was tested with the nonparametric Mann-Whitney or Kruskal-Wallis test (Statgraphics 4.0 software, Manogistics). The significance level was set at $P < 0.05$.

RESULTS

HCMV DNA in breast milk in breastfeeding mothers. To determine the incidence of HCMV shedding during lactation, cell-free milk whey from the breast milk of 73 mothers who gave birth to very preterm infants was screened for HCMV DNA over a period of 1 to 2 months postdelivery. Forty eight of 73 (66%) mothers shed HCMV DNA in their breast milk. This observed incidence corresponds to the mean HCMV seroprevalence of about 60% in the German population. For 28
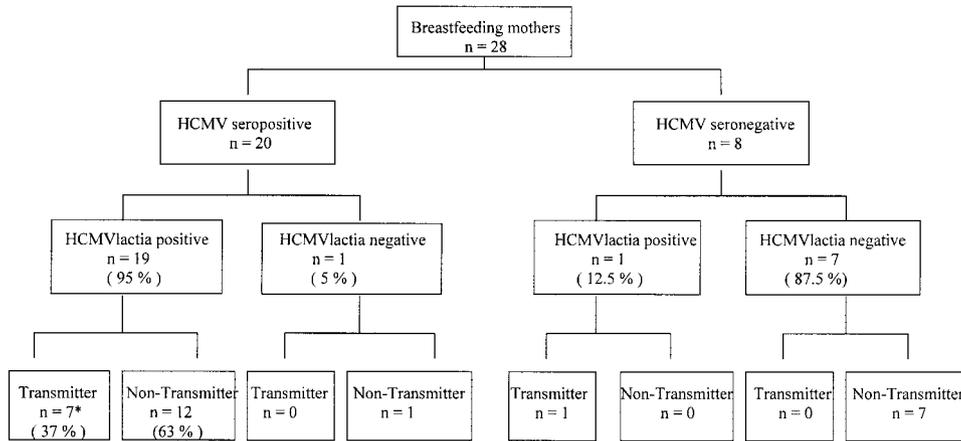
FIG. 1. Incidence of HCMV reactivation and HCMV DNA in breast milk in breastfeeding mothers and the risk of HCMV transmission to their preterm infants. *, One mother transmitted the virus to both twins, resulting in eight HCMV-infected infants. The mode of infection for individual infants remains speculative, only for five of them was postnatal infection confirmed, supporting the hypothesis that HCMV-containing breast milk was the source of infection.

mothers the HCMV serostatus was known and could be compared with the PCR results from breast milk. Nineteen of 20 (95%) seropositive but only one of eight (12.5%) seronegative mothers were positive for HCMV DNA in breast milk ($P > 0.05$; Fig. 1), indicating that in nearly all latently infected women, HCMV is reactivated during lactation.

To test whether HCMV DNA in breast milk coincides with systemic HCMV infection, we tested PBMC from six seronegative and 13 seropositive mothers for HCMV DNA. Of the six seronegative and breast milk-negative mothers, none were found positive for HCMV DNA in blood. Of the 13 HCMV-seropositive and breast milk-positive mothers, only two (15%; one mother IgG^+/IgM^-, the other one IgG^+/IgM^+) were positive for HCMV DNA in PBMC (Table 2). Thus, HCMV infection in lactating mothers seems to be mainly restricted to the breast but not systemic.

HCMV transmission to preterm infants. To calculate the mother-to-child transmission rate of HCMV, all infants were screened for HCMV DNA in the urine and upper respiratory tract. In total, 20 of 48 (42%) HCMV-positive mothers (as determined by HCMV DNA amplification from breast milk) were suspected to have transmitted the virus to their infants. Figure 2 shows a representative Southern blot for selected mother-child pairs. Since 48 HCMV-positive mothers delivered 55 preterm infants and one mother transmitted the virus to both of her twins, the cumulative transmission rate in preterm infants from HCMV-positive mothers was 38% (21 of 55). Referring to all mothers and infants independently from HCMV status, the total transmission rates were 27% (20 of 73)

and 24% (21 of 89) with regard to the mothers and infants, respectively.

Referring to only those mothers for which the HCMV serostatus was known, 7 of 19 (37%) seropositive and breast milk-positive mothers were suspected to have transmitted the virus to their eight infants (one mother transmitted the virus to both twins; Fig. 1). For five of them, postnatal transmission was confirmed. These infants might have been infected by being fed HCMV-containing breast milk.

Postnatal HCMV infection could be confirmed for 12 of the 21 infected infants. In these infants HCMV DNA was detected in urine not earlier than after 2 weeks postpartum and following negative PCR with urine collected during the first 2 weeks (1, 11). For nine infants from nine mothers the mode and time
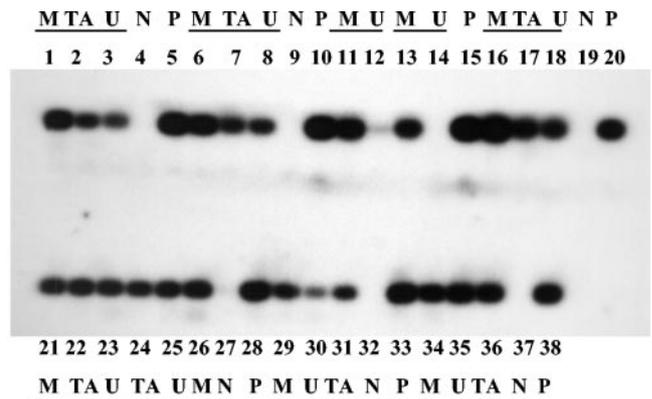

FIG. 2. Autoradiogram of Southern blot analysis of HCMV PCR products. PCR products were separated on a 3% agarose gel, transferred to a nylon membrane, and hybridized with internal oligonucleotide P2 (21) end labeled by T4 polynucleotide kinase and [γ-^{32}P]ATP. Hybridization was carried out for 18 h at 52°C in 5× SSC–5× Denhardt's solution–0.1% sodium dodecyl sulfate. After washing, the filters were exposed to a phosphoscreen. Hybridization signals were visualized by phosphoimaging (PhosphorImager, type SI). P, positive control (0.1 pg of AD169 DNA); N, negative (buffer) control; M, breast milk; TA, tracheal aspirate; U, urine. The PCR products from one mother-child pair are underlined.

TABLE 2. Correlation between HCMV serostatus, HCMV DNA in breast milk, and HCMV DNA in blood in breastfeeding mothers

HCMV serostatus (*n*)	No. with HCMV DNA/total (%)	
	Breast milk	PBMC
IgM^-/IgG^- (6)	0/6 (0)	0/6 (0)
IgM^-/IgG^+ (12)	12/12 (100)	1/12 (8)
IgM^+/IgG^+ (1)	1/1 (100)	1/1 (100)

TABLE 3. Diagnostic and clinical data for the 22 infants with HCMV infection

Infected infant no.	Sex	Birth wt (g)	First detection of HCMV DNA (days postpartum) in		Mode of transmission	Disease noted[a]					HCMV-associated symptoms	Mode of delivery
			Urine	Aspirates		BPD$_1$	BPD$_2$	RDS	ROP	AI		
1	M	1,670	14	14	Postnatal	0	0	0	0	+		Vaginal
2	F	380	28	28	Postnatal	+	+	+	+	+	Sepsis-like disease	Caesarean
3	M	970	28	28	Postnatal	+	+	+	+	0		Caesarean
4	M	815	14	28	Postnatal	+	+	+	+	+		Caesarean
5	F	726	14	14	Postnatal	0	0	0	0	0		Caesarean
6	M	1,080	20	20	Postnatal	+	0	+	0	+		Caesarean
7	M	1,186	14	28	Postnatal	0	0	0	+	+		Vaginal
8	F	930	20	20	Postnatal	0	0	0	0	+		Caesarean
9	F	1,305	24	24	Postnatal	0	0	0	0	+	Hepatitis-cholestase/icterus	Caesarean
10	M	1,670	28	7	Postnatal	0	0	0	0	+		Vaginal
11	F	680	14	28	Postnatal	+	0	+	0	0		Caesarean
12	F	1,480	12	12	Postnatal	0	0	+	0	0		Caesarean
13	F	1,290	12	5	Postnatal	+	+	+	0	0		Caesarean
14	M	960	5	12	Unknown	+	0	+	+	0		Caesarean
15	F	730	3	5	Unknown	0	0	0	+	0		Caesarean
16	F	670	7	5	Unknown	+	+	+	0	0		Caesarean
17	M	2,010	5	3	Unknown	0	0	+	0	+		Vaginal
18	M	1,070	5	3	Unknown	+	0	+	+	+		Caesarean
19	M	1,020	3	3	Unknown	0	0	+	0	0		Vaginal
20	F	960	5	1	Unknown	+	0	+	0	+		Vaginal
21	M	1,320	3	1	Unknown	+	0	0	0	0		Caesarean
22	M	780	3	1	Unknown	0	0	0	0	0		Caesarean

[a] +, positive; 0, negative.

of infection remained unclear as HCMV DNA became detectable in urine during the first 2 weeks of life (Table 3). Notably, among the infants with postnatal HCMV infection, one of the twins (the second child remained unaffected) acquired HCMV in the urine despite no detectable HCMV DNA in the breast milk of the mother during the study period. Since the HCMV serostatus of the mother was unknown, HCMV transmission to this infant might be independent of the mother. For this infant, nosocomial infection, e.g., by transfusion of HCMV-positive blood from a seronegative screened blood donor, cannot be ruled out. Including this child, the number of HCMV-infected infants in our study was 22, 13 of which developed postnatal infection (Table 3).

HCMV-associated disease in preterm infants. In some studies HCMV transmission to preterm infants was found to be associated with a significant risk of developing severe HCMV disease (11, 17). In our study group of 2 of 22 infected infants developed a symptomatic HCMV infection (10%). For both infants postnatal infection was confirmed and both had been fed HCMV-containing breast milk. Sepsis-like symptoms were observed in one infant, and the second infant developed hepatitis with cholestase/icterus for which no reason other than HCMV infection could be confirmed. Despite the fact that there was no significant statistical difference with respect to birth weight and gestational age between the HCMV-infected and noninfected infants (Table 1), the infant developing sepsis-like symptoms had a very low birth weight of 380 g, a gestational age of only 24 weeks, and was the only survivor of triplets. It also developed severe neutropenia and second-stage bronchopulmonary dysplasia (bronchopulmonary dysplasia diagnosed at the 36th postconception week) (Table 3). In contrast to this, the child with hepatitis and cholestase/icterus had

a birth weight of 1,305 g and a gestational age of 31 weeks and did not develop bronchopulmonary dysplasia. Because both infants had severe clinical symptoms and no reason other than HCMV could be confirmed, they were treated with ganciclovir, which resulted in remission of the symptoms. Among the infants positive for HCMV during the first 2 weeks of life, no typical HCMV-associated symptoms were observed (Table 3).

Although the remaining 20 infants with HCMV infection did not demonstrate classical symptoms of HCMV disease, neonatal infection (interleukin-6, ≥100 pg/ml of plasma), retinopathia neonatorum, respiratory distress syndrome due to surfactant deficiency, and bronchopulmonary dysplasia were more frequently observed than in infants without HCMV infection (Table 1). However, these differences were not statistically significant. Most HCMV-infected infants also had to be ventilated for a longer time than those without HCMV infection.

DISCUSSION

Sixty-six percent (48 of 73) of the mothers of preterm infants shed HCMV DNA in their breast milk, which is in agreement with the overall HCMV seroprevalence of 55 to 65% in Germany. The availability of HCMV serostatus data on 28 mothers (eight seronegative and 20 seropositive) allowed us to draw conclusions on HCMV reactivation. HCMV DNA in breast milk was observed in 95% of HCMV antibody-positive mothers (19 of 20), indicating that in nearly all latently infected mothers HCMV underwent reactivation postpartum. Additionally, among the eight mothers who were negative for HCMV antibodies, we found one mother who was also positive for HCMV DNA in breast milk. This finding is in agreement with our previous observation that about 5 to 10% of HCMV-

seronegative blood donors are PCR positive in the peripheral blood (22) and, therefore, virus carrier. In contrast to these observations, other investigators were not able to found HCMV DNA in breast milk from seronegative mothers (11, 30). False negative enzyme-linked immunosorbent assay results and the presence of antibodies not detectable with the assay used are two possible explanations for this phenomenon.

Interestingly, the seronegative but breast milk-positive mother transmitted the virus to her infant. In a similar study using cell-free milk whey, HCMV reactivation was observed in 50% of all mothers and in 96% of HCMV-seropositive mothers (11). Different studies done in Japan with unfractionated breast milk (for which HCMV DNA PCR was found slightly less sensitive [11]) revealed HCMV DNA in breast milk in 40 to 70% of all mothers (despite an overall seroprevalence as high as 90%) and in 67 to 92% of seropositive mothers (2, 14, 20, 32). In summary, our study shows that in all latently infected mothers, HCMV is reactivated during lactation and shed within the breast milk.

The careful studies by Hamprecht et al. (11, 13) indicated that HCMV DNA in breast milk is the consequence of locally restricted virus reactivation in the breast rather than the consequence of a systemic virus reactivation. We observed HCMV DNA in the blood in only 2 of 13 seropositive mothers with HCMV DNA in breast milk, which is in agreement with their results. At present the mechanism and site of HCMV reactivation during lactation are not known. The only definitively known sites of HCMV latency are granulocyte/monocyte/dendritic cell precursors in the bone marrow (10, 15, 19, 27). However, systemic infections were observed very rarely in our study or those done by others. Furthermore, viral load in the cell fraction of breast milk was significantly lower than in the cell-free milk whey (2, 11). These observations may lead to the hypothesis that the human breast harbors latently infected cells or latently infected cells are transported very efficiently into the lactating breast.

In the lactating breast, bioactive substances may be produced or accumulated, which induce or support replication of the virus in its target cells. Using in vitro transfection and infection systems, we were recently able to show that cell-free milk whey is indeed able to stimulate HCMV IE1/2 enhancer/promoter-derived gene expression in monocytic cells (one possible target cell type for HCMV in the lactating breast). This promoter is responsible for initiation of viral replication and, moreover, determines the efficiency of replication. Additionally, milk whey enhanced HCMV replication in permissive human embryonal lung fibroblasts (J. Meier et al., unpublished observations).

In our study, 7 of 19 (37%) HCMV antibody- and breast milk-positive mothers are suspected to have transmitted the virus to their eight infants. Among all 48 breast milk-positive (and therefore HCMV-positive) mothers screened, 42% (20 of 48) are suspected to have transmitted HCMV to their 21 infants, including one mother who transmitted the virus to both twins. In similar studies, transmission was observed in 5 to 37% of HCMV-positive mother-infant pairs (2, 11, 17, 20, 32). The lower transmission rates of 25%, 10%, and 5% observed by Mosca et al. (20), Yasuda et al. (32), and Sharland et al. (26), respectively, may be caused by prophylactic application of intravenous immunoglobulins (20) and freezing of the milk 26,

32) before feeding. In our hospital, breast milk was frozen only sporadically; normally, infants (including preterm babies) were fed untreated breast milk. In addition to the 21 infants from breast milk-positive mothers, there was one twin who became postnatally infected, although the mother was breast milk negative. The reason for this infection is unknown but could be independent of breastfeeding. Including this child, the total number of infected preterm infants in our study group was 22.

For 13 of the 22 HCMV-infected babies, postnatal infection could be confirmed as HCMV DNA became detectable in urine not earlier then 2 weeks after delivery. Infection of these infants (with one exception, see above) was preceded by feeding with HCMV DNA-containing breast milk, supporting that feeding of HCMV-containing milk is a source of HCMV transmission from mother to child. In the other nine infants we found HCMV DNA in urine during the first and second weeks of life. The mode of HCMV transmission to these infants remained unclear, and breastfeeding alone seems not to be responsible for viral transmission. One possibility is perinatal transmission, however, only three of the nine (33%) infants were born vaginally, supporting possible infection by contact with HCMV-contaminated genital secretions. Additionally, perinatal infection seems highly unlikely because in a more recent study on perinatal HCMV transmission, the investigators detected HCMV DNA in only 1 of 24 different cervical swabs (20). Asymptomatic intrauterine infection cannot be ruled out. However, in placenta tissue and umbilical cord blood from five of five infants with unclear modes of infection no HCMV DNA was detected. For a control, HCMV DNA could be amplified from both specimens from a baby with symptomatic prenatal infection, but not from the specimens from 25 noninfected infants (data not shown).

Two of the 13 postnatally infected preterm babies (9% of all 22 infected infants, 15% of infants with verified postnatal infection) developed severe symptomatic HCMV disease with sepsis-like symptoms in one child and hepatitis with cholestase/icterus in the other one. Development of sepsis-like symptoms in one infant correlated with an extremely low birth weight (380 g) supporting that especially preterm infants with very low birth weight have an increased risk for symptomatic HCMV disease following breastfeeding with HCMV-containing milk. Similarly, Hamprecht et al. (11) observed severe symptomatic infection (4 cases of sepsis-like disease) in 12% (4/33) of postnatally infected infants with extremely low birth weight. In other studies in which infants were prophylactically treated with immunoglobulines or fed with pretreated (freezing) milk, symptomatic HCMV disease was not observed (2, 20, 26, 32). However, very recently freezing of breast milk has been found to be not sufficient to prevent HCMV transmission (18). More careful clinical analysis of HCMV-infected infants in the German study group even revealed that half of the infected children (16/33, 48%) had clinical symptoms compatible with HCMV infection (17). Similarly, we observed bronchopulmonary dysplasia, respiratory distress syndrome, amniotic infection, and neonatal infection in half of the HCMV-positive infants. Moreover, the overall incidences of bronchopulmonary dysplasia, respiratory distress syndrome, retinopathia neonatorum and neonatal infection were increased in HCMV-infected infants compared to noninfected infants (41 vs. 33%,

64 vs. 45%, 32 vs. 18% and 50 vs. 39%, respectively), however, these differences were not statistically significant.

In summary, it should be taken in consideration that HCMV becomes reactivated in all latently infected mothers during lactation and is shed within the breast milk. Preterm infants of HCMV-positive mothers are particularly at a high risk of getting infected. At least 10% of infected preterm infants may develop severe clinical symptomatic HCMV disease. In up to 50% of infected infants HCMV may contribute clinical complications commonly observed in preterm infants (e.g., bronchopulmonary dysplasia, respiratory distress syndrome) and may delay development of infants (e.g., maturation of the lung). Our recent studies (4) on the mechanisms involved in HCMV-associated lung pathogenesis support the idea that the virus, by inhibiting expression of the epidermal growth factor receptor, may contribute to delayed lung maturation and impaired function of the surfactant system. This finding correlates with the observation that HCMV was present in the respiratory tract of all infected preterm infants and that the time of assisted respiration for HCMV-infected infants was higher than for noninfected infants (23; this paper).

Considering that in Germany at least 10% of HCMV-infected preterm infants become severely ill following HCMV infection during the early postnatal period methods for efficient inactivation of the virus in the breast milk without impairment of its immunological and nutritional constituents should be developed and introduced in clinical routine. As the risk of developing HCMV disease is particularly high in preterm infants with very low birth weights, we would like to recommend that mothers initially treat the breast milk given to very preterm infants born before and during the 28th week of gestation. The time period for feeding pretreated breast milk can be limited to the first 6 weeks, according to the findings of Hamprecht et al. (11), who observed significant DNA in breast milk up to day 40 postpartum. This period should be adapted individually for infants who can be breastfed. Similarly, the Austrian Society of Pediatrics recommended pasteurization of breast milk for all HCMV-seropositive mothers until gestational week 34 to minimize the risk of postnatal HCMV transmission (33). Because our study also demonstrated that serological methods alone may not be sufficient to identify all potential HCMV transmitting mothers, treatment of breast milk should be performed independently of the serostatus of the women.

REFERENCES

1. **Alford, C. A., S. Stagno, R. F. Pass, and W. J. Britt.** 1990. Congenital and perinatal cytomegalovirus infections. Rev. Infect. Dis. **12:**S745–S753.
2. **Asanuma, H., K. Numazaki, N. Nagata, T. Hotsubo, K. Horino, and S. Chiba.** 1996. Role of milk whey in the transmission of human cytomegalovirus infection by breast milk. Microbiol. Immunol. **40:**201–204.
3. **Ballard, R. A., W. L. Drew, K. G. Hufnagle, and P. A. Riedel.** 1979. Acquired cytomegalovirus infection in preterm infants. Am. J. Dis. Child. **133:**482–485.
4. **Bauer, H., Y. Ting, C. E. Greer, J. C. Chambers, C. J. Tashino, J. Chimera, A. Reingold, and M. M. Manos.** 1991. Genital human papilloma virus infection in female university students as determined by a PCR-based method. JAMA **265:**472–477.
5. **Beutler, T., C. Höflich, S. A. Stevens, D. H. Krüger, and S. Prösch.** 2003. Downregulation of the epidermal growth factor receptor by human cytomegalovirus infection in human fetal lung fibroblasts. Am. J. Respir. Cell. Mol. Biol. **28:**86–94.
6. **Bryant, P., C. Morley, S. Garland, and N. Curtis.** 2002. Cytomegalovirus transmission from breast milk in premature babies: does it matter? Arch. Dis. Child Fetal Neonatal **87:**F75–F77.
7. **Döcke, W. D., S. Prösch, E. Fietze, V. Kimel, H. Zuckermann, C. Kluge, U. Syrbe, D. H. Krüger, R. von Baehr, and H. D. Volk.** 1994. Cytomegalovirus reactivation and tumor necrosis factor. Lancet **343:**268–269.
8. **Dworski, M., M. Yow, S. Stagno, R. F. Pass, and C. Alford.** 1983. Cytomegalovirus infection of breast milk and transmission in infancy. Pediatrics **72:**295–299.
9. **Fietze, E., S. Prösch, P. Reinke, J. Stein, W. D. Döcke, G. Staffa, S. Loening, S. Devaux, F. Emmerich, R. von Baehr, D. H. Krüger, and H. D. Volk.** 1994. Cytomegalovirus infection in transplant recipients: role of tumor necrosis factor. Transplantation **58:**675–680.
10. **Hahn, G., R. Jores, and E. S. Mocarski.** 1998. Cytomegalovirus remains latent in a common precursor of dendritic and myeloid cells. Proc. Natl. Acad. Sci. USA **95:**3937–3942.
11. **Hamprecht, K., J. Maschmann, M. Vochem, K. Dietz, C. P. Speer, and G. Jahn.** 2001. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. Lancet **357:**513–518.
12. **Hamprecht, K., M. Vochem, A. Baumeister, M. Boniek, C. P. Speer, and G. Jahn.** 1998. Detection of cytomegaloviral DNA in human milk cells and cell free milk whey by nested PCR. J. Virol. Methods **70:**167–176.
13. **Hamprecht, K., S. Witzel, J. Maschmann, C. P. Speer, and G. Jahn.** 2000. Transmission of cytomegalovirus infection through breast milk in term and preterm infants, p. 231–239. *In* B. Loletzko (ed.), Short and long term effects of breastfeeding on child health. Kluwer Academic/Plenum Publishers, Berlin, Germany.
14. **Hotsubo, T., N. Nagata, M. Shimada, K. Yoshida, K. Fujinaga, and S. Chiba.** 1994. Detection of human cytomegalovirus DNA in breast milk by means of polymerase chain reaction. Microbiol. Immunol. **38:**809–811.
15. **Kondo, K., J. Xu, and E. S. Mocarski.** 1996. Human cytomegalovirus latent gene expression in granulocyte-macrophage progenitors in culture and in seropositive individuals. Proc. Natl. Acad. Sci. USA **93:**11137–11142.
16. **Kumar, M. L., G. A. Nankervis, I. B. Jacobs, C. B. Ernhart, C. E. Glasson, P. M. McMillan, and E. Gold.** 1984. Congenital and postnatally acquired cytomegalovirus infections: long term follow up. J. Pediatr. **104:**674–679.
17. **Maschmann, J., K. Hamprecht, K. Dietz, G. Jahn, and C. P. Speer.** 2001. Cytomegalovirus infection of extremely low-birth weight infants via breast milk. Clin. Infect. Dis. **33:**1998–2003.
18. **Maschmann, J., C. P. Speer, G. Jahn, and K. Hamprecht.** 2002. Freezing of breast milk does not prevent Cytomegalovirus (CMV) infection in premature infants: a case report and results from spiking experiments. J. Matern. Fetal Neonatal Med. **11**(Suppl. 1)**:**110 (abstract).
19. **Mendelson, M., S. Monard, P. Sissons, and J. Sinclair.** 1996. Detection of endogenous human cytomegalovirus in CD34+ bone marrow progenitors. J. Gen. Virol. **77:**3099–3102.
20. **Mosca, F., L. Pugni, M. Barbi, and S. Binda.** 2001. Transmission of cytomegalovirus. Lancet **357:**1800.
21. **Olive, D. M., M. Simsek, and S. Al-Mufti.** 1989. Polymerase chain reaction assay for detection of human cytomegalovirus. J. Clin. Microbiol. **27:**1238–1242.
22. **Prösch, S., V. Kimel, I. Dawydowa, and D. H. Krüger.** 1992. Monitoring of patients for cytomegalovirus after organ transplantation by centrifugation culture and PCR. J. Med. Virol. **38:**246–251.
23. **Prösch, S., U. Lienicke, C. Priemer, G. Flunker, W. F. Seidel, D. H. Krüger, and R. R. Wauer.** 2002. Human adenovirus and human cytomegalovirus infections in preterm newborns: no association with bronchopulmonary dysplasia. Pediatr. Res. **52:**219–224.
24. **Prösch, S., H. D. Volk, P. Reinke, K. Pioch, W. D. Döcke, and D. H. Krüger.** 1998. Human cytomegalovirus infection in transplant recipients: Role of TNFα for reactivation and replication of human cytomegalovirus, p. 29–34. *In* M. Scholz, H. F. Rabenau, H. W. Doerr, and J. Cinatl (ed.), CMV-related immunopathology, Monographs in Virology, vol. 21. Karger, Basel, Switzerland.
25. **Prösch, S., C. E. C. Wendt, P. Reinke, C. Priemer, M. Oppert, D. H. Krüger, H. D. Volk, and D. W. Döcke.** 2000. A novel link between stress and human cytomegalovirus (HCMV) infection: sympathic hyperactivity stimulates HCMV activation. Virology **272:**357–365.
26. **Sharland, M., M. Khore, and A. Russell-Bedford.** 2002. Prevention of postnatal cytomegalovirus infection in preterm infants. Arch. Dis. Child. Fetal Neonatal **86:**F140.
27. **Sindre, H., G. E. Tjoonnfjord, H. Rollag, T. Ranneberg-Nilsen, O. P. Veiby, S. Beck, M. Degre, and K. Hestdal.** 1996. Human cytomegalovirus suppression of and latency in early hematopoietic progenitor cells. Blood **88:**4526–4533.
28. **Stagno, S., D. W. Reynolds, R. F. Pass, and C. A. Alford.** 1980. Breast milk and the risk of cytomegalovirus infection. N. Engl. J. Med. **302:**1073–1076.
29. **Stagno, S., and R. J. Whitley.** 1985. Herpesvirus infections of pregnancy. Part

I: Cytomegalovirus and Epstein-Barr virus infections. N. Engl. J. Med. **313:** 1270–1274.

30. **Vochem, M., K. Hamprecht, G. Jahn, and C. P. Speer.** 1998. Transmission of cytomegalovirus to preterm infants through breast milk. Pediatr. Infect. Dis. J. **17:**53–58.

31. **Yaeger, A. S., P. E. Palumbo, N. Malachowski, R. I. Ariagno, and D. K. Stevenson.** 1983. Sequelae of maternally derived cytomegalovirus infections in preterm infants. J. Pediatr. **102:**918–922.

32. **Yasuda, A., H. Kimura, M. Hayakawa, M. Ohshiro, Y. Kato, O. Matsuura, C. Suzuki, and T. Morishima.** 2003. Evaluation of cytomegalovirus infections transmitted via breast milk in preterm infants with a real-time polymerase chain reaction assay. Pediatrics **11:**1333–1336.

33. **Zwiauer, K., J. Deutsch, U. Goriup, H. Haas, N. Haiden, H. Holzmann, B. Pietschnig, A. Pollak, T. Popow, I. Rock, W. Sperl, and K. Widhalm.** 2003. Prävention von Muttermilch-mediierten CMV-Infektionen bei Frühgeborenen. Monatszeitschrift Kinderheilkunde **12:**1346–1347.