

Cytomegalovirus infection via mother's milk: could distinct virus strains determine different disease patterns in preterm twins?

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SUMMARY

We describe a case of cytomegalovirus infection via the mother's milk in preterm twins who showed a different disease pattern. Molecular analyses to genotype the viral genome disclosed a mixed population of virus strains in the milk. These isolates were differentially transmitted to each of the twins and this may be responsible of the different patterns of disease.

KEY WORDS: Cytomegalovirus infection, Virus strains, Mother's milk, Very low birth weight infants, Twins

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CASE REPORT

Preterm dizygotic male twins were born by elective cesarean section for maternal pre-eclampsia at 28 weeks and 5 days' post-conceptual age to a 39-year-old primipara.

The mother was found immune for cytomegalovirus (CMV) during the first trimester of pregnancy, datum confirmed 24 hours after delivery. Congenital infection was ruled out in both twins by virus isolation at birth (Lanari *et al.*, 2006).

In accordance with the surveillance protocol for preterm babies, the fresh mother's milk was tested weekly to detect and quantitate CMV-DNA (Lanari *et al.*, 2006). The first sample of breast

milk was collected on the 3rd day after delivery and was negative for virus isolation, whereas the second sample collected on the 10th day after delivery (at 30 weeks' post-conceptual age) was still negative for virus isolation but positive for qPCR (Table 1).

Subsequent samples were positive for both tests and the viral load in breast milk increased until 6 weeks after delivery (Table 1).

Of the two babies, the first-born had a birth weight of 1370 g (50thp) and his Apgar score was 7 and 9 at 1 and 5 minutes, respectively. He needed endotracheal surfactant administration and nasal continuous positive air pressure (nCPAP) for respiratory distress syndrome, ibuprofen for closing patent ductus arteriosus, and a filtered-irradiated red blood cell transfusion from a CMV-seronegative donor at 32 weeks post-conceptual age for anem. Enteral feeding was started 3 days after birth and 10 days later the baby reached the full diet with mother's milk fresh or refrigerated at 4°C for 24 hours. At 33 weeks' post-conceptual age he showed an isolated desaturation spell, without any other clinical abnor-

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TABLE 1 - Clinical and laboratory findings in mother and twins.

Post-conceptual age (Chronologic age) weeks	Mother gN-4c/gB-2 and gN-1/gB-2		First born gN-4c/gB-2		Second born gN-1/gB-2	
	Breast milk		Urine	Blood	Urine	Blood
	CMV isolation	CMV qPCR copies/mL	CMV isolation	Neutrophil count per mm ³	CMV isolation	Neutrophil count per mm ³
29 (1)	-	<600	-	nd	-	nd
30 (2)	-	5.4 x 10 ³	-	2608	-	nd
31 (3)	+	1.1 x 10 ⁴	-	3177	-	3699
32 (4)	+	1.1 x 10 ⁴	-	1759	-	2785
33 (5)	+	1.5 x 10 ⁴	-	1198	-	nd
34 (6)	+	3.8 x 10 ⁴	-	nd	-	1474
35 (7)	-	nd	+	1420	-	nd
36 (8)	-	1.3 x 10 ⁴	+	nd	-	nd
37 (9)			nd	1273	+	1143
48 (20)	No production		+	401	+	1283
50 (22)			nd	1422	nd	1412
52 (24)			+	1550	+	1670

Symptoms: repeated desaturation spells, poor skin perfusion, tachycardia, tachypnea, distended abdomen, hyporeactivity (sepsis-like syndrome). qPCR: quantitative Polymerase Chain Reaction. + = positive result; - = negative result; nd = not determined.

malities except for an absolute neutrophil count of 1198/mm³. At that time the CMV isolation in urine was still negative, but he resulted positive for viral DNA for the first time. At 35 weeks' post-conceptual age he showed the clinical pattern of sepsis-like syndrome (Hamprrecht *et al.*, 2001). White blood cells were 8880/mm³ and neutrophils were 1420/mm³, while haemoglobin, platelets, hepatic and renal function, C reactive protein and urine analysis, chest X-ray, cerebral and cardiac ultrasonography were all normal. Blood culture was negative for bacteria and mycetes, but CMV isolation in urine was positive for the first time. He recovered from the sepsis-like syndrome in 48 hours but a mild/severe neutropenia persisted for many weeks (Table 1).

The second-born twin had a birth weight of 710g (<3rdpercentile) and his Apgar score was 6 and 8

at 1 and 5 minutes, respectively. He required nCPAP for mild respiratory distress and a filtered-irradiated red blood cell transfusion for anemia at 30 weeks' post-conceptual age from a CMV-seronegative donor. Enteral feeding was started 3 days after birth and at 12 days of life full enteral feeding was reached with fresh or refrigerated mother's milk. Weekly testing for CMV in urine was negative until 37 weeks' post-conceptual age when CMV was detected for the first time. The only abnormal finding was a mild neutropenia which started at 34 weeks' post-conceptual age and persisted until 50 weeks' post-conceptual age. CMV-DNAemia was positive during this period.

Breast-feeding lasted until 36 weeks' post-conceptual age for both twins, when the mother's milk production stopped.

To verify whether CMV had been transmitted to the babies through the mother's milk, a genetic analysis of virus variants was performed using the polymorphic genes UL73-gN (seven genotypes: gN-1, gN-2, gN-3a, gN-3b, gN-4a, gN-4b, gN-4c) and UL55-gB (four genotypes at cleavage site: gB-1, gB-2, gB-3, gB-4) to assign a viral genotype (Pignatelli *et al.* 2001 and 2003a). A very sensitive nested gN PCR-assay (Pignatelli *et al.*, 2006) on 300 ng of total DNA was used to amplify UL73-gN from whole milk and twins' urine, while UL55-gB cleavage site was PCR-amplified as previously described (Pignatelli *et al.*, 2001). gB and gN were genotyped by sequencing at Primm Laboratories (Milano, Italy) and confirmed by Restriction Fragment Length Polymorphisms (RFLP) analysis as previously reported (Pignatelli *et al.*, 2003a).

A total of 11 samples (3 samples from the mother's milk and 4 blood and urine samples for each son) chosen during the follow-up were analysed by gN and gB genotyping. A different gN genotype was repeatedly detected in each twin (the first-born was gN-4c and the second-born gN-1), while the mother's milk showed a concomitant shedding of CMV variants gN-4c and gN-1 (Table 1). By contrast, the same gB type 2 was found both in mother's milk and in twins' urine. Both infants were discharged at 38 weeks' post-conceptual age.

Clinical examination, cerebral ultrasonography, funduscopy examination, developmental test and auditory brainstem evoked response were still normal at 88 weeks' post-conceptual age (60 weeks' chronologic age).

Postnatal CMV infection is usually asymptomatic in term babies, while preterm infants, particularly very low weight infants (VLBW; birth weight <1500 g), are more susceptible to symptomatic CMV infection (Yasuda *et al.*, 2003). Neutropenia is the most common manifestation, followed by thrombocytopenia, hepatitis, pneumonia, necrotising enterocolitis and neurologic symptoms, and sepsis-like syndrome (Hamprecht *et al.*, 2001; Maschmann *et al.*, 2001; Stranska *et al.*, 2006).

The main source of postnatal CMV transmission in both term and preterm infants is breast milk, although postnatal CMV infection due to infected maternal cervical secretions, transmission by person-to-person spread or via blood transfusion are less commonly possible (Schleiss, 2006).

In the industrialized countries from 40% to 80% of pregnant women are CMV-seropositive (Gaytant *et al.*, 2002). Nevertheless, between 66 and 93% of them excrete CMV DNA in their breast milk (Schleiss, 2006) and 76% shed the whole virus (Hamprecht *et al.*, 2001). Between 37 and 59% of preterm infants fed with CMV infected fresh milk are infected (Schleiss, 2006).

In agreement with literature data, we found that the collection of mother's milk at close intervals allowed us to ascertain that CMV DNA was detectable in breast milk from the first week after delivery, while the virus excretion started later. Moreover, we found that CMV load in the milk reached a peak 6 weeks after delivery and thereafter the two babies started excreting CMV in the urine.

Even though the twins were exposed to the same infective source at the same time and with the same viral load, they had a different course in terms of infection latency and clinical manifestations. Interestingly, neutropenia was seen two weeks before CMV urine excretion began in both infants and was long-lasting, but only the first twin had evident clinical manifestations of illness. No long-term *sequelae* linked to CMV infection were present in either twin at 15 months' follow-up (60 weeks' chronologic age).

We detected two different CMV strains in the mother's milk as previously reported (Prix *et al.* 1998; Stranska *et al.*, 2006). However, each of the twins seems to have acquired, at least predominantly, only one strain which was presumably responsible for each pattern of clinical manifestations.

According to the literature, the different virulence among CMV clinical isolates might be an important factor in determining the occurrence and severity of CMV disease (Pignatelli *et al.*, 2004). This could depend on genetic variation detected among wild type strains in genes involved in several mechanisms of CMV biology and immunopathogenesis. gN is one of the most polymorphic envelope glycoproteins and its seven genotypes (gN-1, gN-2, gN-3a, gN-3b, gN-4a, gN-4b, gN-4c - Pignatelli *et al.*, 2003a) have been hypothesized to have different virulence. In particular, the data reported so far analysing CMV-seropositive healthy blood donors (Pignatelli *et al.*, 2006), AIDS patients (Pignatelli *et al.*, 2003b), solid organ transplant recipients (Rossini *et al.*,

2005) and CMV congenitally-infected newborns (Pignatelli *et al.*, submitted for publication), suggest that gN-1 strains are less virulent than the variants belonging to gN-4 group.

In our case, the first twin infected with genotype gN-4c was the only one who showed evident clinical manifestations of illness, while the second one, infected with a gN-1, showed no symptoms attributable to CMV infection. This finding supports the hypothesis of a different virulence of gN variants, since the discrepancy in severity of CMV disease could be presumably ascribed to the distinct genomic variants detected.

Human milk is the best nutritional choice for newborns especially important for preterm infants, whose immune system is immature. According to the American Academy of Pediatrics decisions (2005) about breast-feeding VLBW infants by mothers known to be CMV-seropositive should be made considering the potential benefits of human milk versus the risk of CMV transmission.

Freezing breast milk preserves the biochemical and immunological quality of the milk but can only decrease the CMV viral load, while traditional pasteurization (30 min at 62.5°C) destroys viral infectivity (Hamprecht *et al.*, 2004), but also alters the biochemical and immunological quality. Hence neither of the treatments are optimal.

Therefore, using a molecular marker of pathogenicity, and gN genotypes seem to be a good candidate for this purpose, to establish shedding of a CMV strain at increased virulence via the mother's milk could add further information on the risk of a symptomatic CMV infection and thereby influence the choice of a specific milk treatment for VLBW infants.

More uniform and conclusive recommendations for the prevention of virus transmission in VLBW infants are needed and further studies on potential CMV markers of virulence and pathogenicity should be strongly encouraged.

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