

Review

Human cytomegalovirus infections in premature infants by breastfeeding

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Human cytomegalovirus (CMV) is the most common cause of congenital and perinatal infections. Understanding the epidemiology of CMV is a key element in development of strategies for prevention of infection in premature infants. Breast-fed infants are susceptible to CMV infection from breast milk. CMV was isolated more frequently from breast milk at more than one month after delivery than from colostrum or early breast milk. CMV particle shedding into milk whey have a more important role. Cytokines in serum and milk are related to the reactivation of CMV, which occurs locally in the mammary gland of the lactating mother after delivery. Premature infants with low concentration of serum antibodies can acquire CMV infection from the fresh breast milk containing the virus. Freezing breast milk may be protective for the preterm infant until the titer of CMV antibody increases. However clinical importance of CMV infection in premature infants by breast-feeding is still unclear. This mini-review focuses on recent advances in the study of CMV infection in premature infants by breast-feeding.

Key words: cytomegalovirus (CMV), premature infants, breastfeeding, reactivation.

INTRODUCTION

Human cytomegalovirus (CMV) is the most common cause of congenital and perinatal infections throughout the world.

The prevalence of congenital CMV infection varies widely between different populations (0.2-3.0%). Only less than 5% of the infants with congenital CMV infection have typical clinical symptoms of cytomegalic inclusion disease (CID), another 5% have atypical involvement, and the remainder (90%) is asymptomatic at the time of delivery (Numazaki and Chiba, 1997). Even asymptomatic at birth, 5 to 17% of infants with these asymptomatic congenital CMV infections will develop progressive sensorineural hearing loss or other neurodevelopmental difficulties within first 4 years of life after birth (Numazaki et al., 2002; Numazaki and Fujikawa, 2004).

We previously reported the incidence of congenital CMV infection in Japan (Numazaki and Chiba, 1996). Of 7, 995 Japanese neonates, 31 (0.39%) were identified as having congenital CMV infections on the basis of viuria at birth. Three of 31 infants had clinically severe disease resulting in death during the neonatal period. As

decrease in the prevalence of serum antibodies against CMV has been speculated in recent years in the last 20 years (Nishimura et al., 1999), the incidence of primary infection during pregnancy may be increased in future.

Transmission of CMV by natural routes relates importantly to preventing CMV transmission to the seronegative pregnant women. CMV is isolated more frequently from cervical secretion and semen than from urine and other clinical specimens. Evidences for sexual transmission of CMV were provided by determining prevalence of serum antibodies to CMV and viral shedding in male sex partners of women with and without CMV infection (Numazaki et al., 2000). However, it is also necessary to take into account other potential sources of CMV infection including contact with asymptomatic young children who are excreting CMV at the places such as child care arrangements.

Primary infection of CMV during pregnancy was associated with an increased risk of developmental or intellectual deficit in the offspring. Although CMV can be transmitted to the fetus even if there is preconceptional maternal immunity, reinfection or reactivated latent

infection might be an important determinant of developmental and intellectual impairment in the offspring. The population of seropositive women of childbearing age in low socioeconomic community is about 85% and about 55% in populations of high socioeconomic status. In certain countries parental interest groups have called for screening programs for the general obstetric population in an attempt to reduce the rate of fetal damage with congenital CMV infection.

Although social and economic conditions have improved dramatically, it was also reported that the prevalence of CMV was stable from 1976 to 1990 (Hirota et al., 1992). The prevalence of serum antibodies to CMV was decreased and primary CMV infection during pregnancy was speculated to be increased in Sapporo, Japan during the time of 1988 to 2000 (Numazaki and Fujikawa, 2002). From the results of the recent study, incidences of congenital CMV infection in Japan are estimated to be changed (Numazaki and Fujikawa, 2004).

Breast-fed infants are susceptible to CMV infection from breast milk. Premature infants with low concentration of serum antibodies can acquire CMV infection from the fresh breast milk containing the virus. Freezing breast milk may be protective for the preterm infant until the titer of CMV antibody increases.

However clinical importance of CMV infection in premature infants by breast-feeding is not completely clarified. This mini-review focuses on recent advances in the study of CMV infection in premature infants by breast-feeding relevant for clinicians.

Epidemiology of perinatal CMV Infections

As a result of transmission during the course of delivery and by ingestion of infected breast milk, perinatal infections are much more prevalent than congenital infections. Perinatal CMV infection often involves the hepatobiliary tract but does not usually cause clinical manifestations in normal individuals. Seropositivity for antibodies against CMV is indicative of latent infection, but insufficient as a predictor for the risk of recurrence. In seronegative preterm infants it has been possible to prevent postnatal CMV transmission by screening blood products for CMV and treating banked breast milk (Diosi et al., 1967). The reported rate of transmission for infants fed with CMV-positive breast milk ranges from 58 to 76% (Hayes et al., 1972; Dworsky et al., 1983; Hotsubo et al., 1994).

Liver dysfunction associated with perinatal CMV infections is often recognized in both normal and immunocompromised hosts and in patients with both primary and reactivated CMV infections. Although infantile CMV hepatitis was speculated to be caused by primary infection in the perinatal period, immunological conditions of the hosts may modify the clinical manifestations. We investigated the role of peripheral blood mononuclear

cells, especially CD4+ and CD8+ T lymphocytes, in infants with liver dysfunction associated with perinatal primary CMV infection, by flow cytometry and the polymerase chain reaction (PCR) (Numazaki et al., 1994). Expression of CMV antigens in CD4+ and CD8+ cells was also found in patients with liver dysfunction associated with perinatal primary CMV infection. CMV infection of CD4+ and CD8+ cells may play an important role in the pathogenesis of activation of CMV infection (Fujikawa et al., 2003a, b).

CMV infections by breastfeeding

Since Diosi et al. (1967) succeeded in isolating CMV from breast milk, breast milk has been considered as one of the most important sources of mother-to-infant infection. Hayes et al. (1972) isolated CMV from breast milk of 17 out of 64 seropositive women (27%) and most of the isolates were obtained after the first week. Stagno et al. (1980) reported that breast-fed infants are more frequently infected with CMV than bottle-fed infants by the result of isolation from urine. Dworsky et al. (1983) reported that consumption of infected breast milk led to infection in 69% of infants.

The presence of CMV in breast milk was more frequently observed than in other sites such as vaginal secretion, urine and saliva. Isolation of CMV from colostrum around the time of delivery showed a lower incidence of viral isolation than breast milk at more than one month after delivery. Breastfeeding seemed to be associated more closely with vertical infection than contact with an infected genital tract. Infants who were fed on breast milk for over one month were infected more frequently, and the incidence of infection in infants was significantly higher when the infants were fed by mothers who shed CMV into their milk (Dworsky et al., 1983).

We compared the rates of CMV isolation from breast milk at different times after delivery. Our data support the results of previous studies (Hayes et al., 1972; Dworsky et al., 1983; Hotsubo et al., 1994; Ahlfors et al., 1985) which show that virus excretion into colostrum and milk occurs less frequently in the period a few weeks after delivery. Our results of the detection of CMV immediate early (IE) DNA (Asanuma et al., 1996) also support the data of isolation.

Colostrum and early milk were previously reported to contain abundant IgA and IgM that might be capable of neutralizing CMV during the first few days of lactation (Goldman et al., 1982). However, IgA and IgM antibodies against CMV are not associated with diminished CMV shedding in colostrum and early milk, as CMV DNA has not been detected in colostrum and early milk (Asanuma et al., 1996).

Although lactoferrin and other iron-binding proteins present in colostrum and milk also have bacteriostatic and anti-CMV activity *in vitro* (Harmsen et al., 1995), *in*

vivo roles of these antiviral agents in neonatal and maternal infections has yet to be clarified. The synergistic interaction between sIgA and iron-binding proteins such as lactoferrin has been speculated to have an important role in such defense (Skansn-Saphir et al., 1993). As viral DNA was not detected from colostrum and no anti-CMV effects of liquid supernatant of colostrum was shown, inhibitory effect of antibodies in colostrum was not proved (Numazaki et al., 1996).

Most of the viruses in the human herpesvirus family are transmitted by cell-to-cell contact. Cell-to-cell contact is also the main method of vertical transmission for human T-lymphotropic virus type-I (HTLV-I) and human immunodeficiency virus type-1 (HIV-1) (Kinoshita et al., 1984; Van de Perre et al., 1993). For most viruses including CMV, although transmission has been documented, no serious illness or clinical symptoms in the neonate secondary to breast-feeding has been reported (Numazaki, 1997).

Human breast milk contains many different types of cells associated with immune reactions. Although CMV DNA was detected in milk cells, the rate of detection in whey was higher than in milk cells. CMV particle shedding into whey may have a more important role in vertical infection by breast milk than cell-to-cell transmission. The excretion of CMV into breast milk was not considered to be the primary CMV infection of mothers.

Mononuclear cells of human breast milk have a potential for production of many different cytokines including tumor necrosis factor (TNF)-(α), and interferon (IFN)-(γ) (Goldman et al., 1982; Skansen-Saphir et al., 1993). It is likely that specific cellular interactions as well as other cytokines are necessary for CMV reactivation (Numazaki et al., 1998; Asanuma et al., 1995). In the active phase of CMV infection, serum titers of sIL-2R were correlated with clinical findings.

In postpartum women, the state of cellular immunity is thought to be similar to the state in late pregnancy. The suppression of cellular immunity is thought to induce a localized reaction in the mammary gland and to induce a large amount of CMV shedding into the colostrum. It was suggested that presence of cytokines such as sIL-2 in serum was also related to the reactivation of CMV which occurs locally in the mammary gland of the lactating mother after delivery.

We also tried to evaluate anti-CMV properties and roles of cytokines in human colostrum and breast milk (Numazaki et al., 1997). Anti-CMV activity of colostrum was evaluated by indirect immunofluorescence assay using CMV AD169 strain-infected MRC-5 cells. We measured TNF-(α) and IFN-(γ) activities in breast milk.

Liquid supernatant of colostrum without cytotoxicity was not found to exert inhibitory effect on CMV-infected MRC-5 cells. The activities of TNF-(α) were detected in CMV DNA-negative colostrum and breast milk. These activities were not detected from CMV DNA-positive

milk. IFN-(γ) activities were also detected in colostrum. It is likely that presence of cytokines such as TNF-(α) and IFN-(γ) in colostrum and early breast milk are related to inhibit the reactivation of CMV which occurs locally in the mammary gland of the lactating mother after delivery.

CMV infection in premature infants

CMV excretion into urine is observed between days 30 and 120, a time during which most infants with very low birth weight are still hospitalized and are susceptible to respiratory or other acute infections. Early onset of CMV infection occurred only in extremely immature, preterm infants, and it was associated with a symptomatic course (Hamprecht et al., 2001). Perinatal CMV infection often involves the hepatobiliary tract but not usually cause clinical manifestations. The symptoms were almost similar to previous descriptions of groups of neonates (Dworsky et al., 1982; Kumar et al., 1984).

Symptomatic congenital infections by CMV usually occur in only 0.01% to 0.04% of all newborns. As demonstrated by Prosch et al. (2002), the total incidence of CMV in preterm infants was 18%. Sawyer et al. (1987) as well as Vochem et al. (1998) observed CMV infection in 33% and 25% of preterm infants, respectively. Using the more insensitive method of CMV isolation in cell culture, Yeager et al. (1983) found a CMV incidence of 17%. Hamprecht et al. (2001) observed postnatal CMV infections in 37% (33/90) of preterm infants from seropositive, breastfeeding mothers. In all these studies, the overall rate of CMV infection in preterm infants was higher.

The clinical outcome of CMV infection in preterm newborns is variable, ranging from asymptomatic infection to fatal life-threatening diseases, such as sepsis-like disease (Kumar et al., 1984). However, a recent attempt to prevent maternal and nosocomial CMV transmission from occurring in premature neonates by administering intravenous immunoglobulins failed (Snydman et al., 1995).

Association with chronic lung diseases

Relationship between bronchopulmonary dysplasia (BPD) and congenital infection by pathogens such as *Ureaplasma urealyticum*, *Chlamydia trachomatis*, *Mycoplasma hominis*, or CMV has been speculated (Sawyer et al., 1987; Pierce and Bancalari, 1995; Numazaki et al., 1986; Iles et al., 1996; Wang et al., 1995). Sawyer et al. (1987) reported an association between CMV infection and BPD. Infants with CMV infection, especially those with prenatal and postnatal infection, were significantly longer on ventilation than those without infection. The incidence of chronic lung diseases (CLD) in pre and postnatally infected infants is

higher compared with those infants for which the time of infection remained unclear. All of the infants with the clinical symptom complex had underlying CLD and all had received multiple blood transfusions during their hospitalization (Ballard et al., 1979). Acquired CMV may be relatively common in sick preterm infants and should be distinguished from other causes of rapid deterioration.

CMV frequently may cause active infection in preterm infants. CMV can colonize the upper respiratory tract. CMV may increase the risk of developing CLD including BPD in individual patients, especially in very immature infants. CMV induce early lung inflammation (Grundy et al., 1987) associated with increased expression of proinflammatory cytokines and chemokines. CMV may also trigger inflammatory processes in the immature lung, supporting the development of CLD such as BPD. The pro inflammatory cytokine TNF- stimulates expression of CMV immediate early (IE) proteins which are known to trigger inflammatory processes. Thus, active CMV infection may not only promote development of BPD but, in turn, CMV replication may be enhanced in the BPD lung by an inflammatory process.

Association with breast milk and breastfeeding

If breastfed preterm infants may be more likely than term infants to have asymptomatic CMV infection, preterm infants born vaginally acquired CMV infection also may develop symptomatic infection. Breastfed preterm infants without enough serum titers of transplacental antibodies to CMV may be more likely to have a symptomatic infection. It was suggested that about 40% of the breast-fed children acquire CMV via breast milk and breastfeeding during the first year of their lives (Minamishima et al., 1994). This mother-to-infant transmission of CMV may have certain protective effects on congenital CMV disease in the offspring. However, it was also estimated that infants who are not breastfed have a six fold greater risk of dying from infectious diseases in the first 2 months of life than those who are breastfed in less developed countries.

After preterm infants who were CMV-seronegative were fed banked human milk that was either pasteurized or frozen, no infections were observed (Wang et al., 1995). Pasteurization and freezing to -20°C for 3 days inactivated CMV in naturally infected raw human milk (Friis and Anderson, 1982; Welsh et al., 1979; Goldblum et al, 1984; Speer et al., 1986). This procedure may inactivate CMV in human milk without affecting the nutritional and immunological qualities of human breast milk.

Although one might conceivably remove cell associated virus by filtering, free viral particles are difficult to eliminate. Pasteurization to 62.5°C will destroy infectious viral particles, but this also alters milk composition to a significant degree, and In practical

terms is often limited by the requirement for scrupulous hygiene (Lawrence, 1999; Wright and Feeney, 1998).

Immunological factors may be associated with the pathogenesis of neurological and other sequelae in CMV-infected infants (Numazaki et al., 2002). It is possible that progression of neurologic complications is related to the persistent viral infection and replication of CMV or host immunological response to infection. Protective mechanisms of the innate and cellular immune system at work during lactation could potentially be exploited by vaccination. Most of seropositive breastfeeding mothers had selective reactivation of CMV in their breast milk with an incidence of acquired CMV infection in the neonatal unit. The rate of CMV acquisition in the neonatal unit appears to be high in which did not take preventive measures against CMV.

Hamprecht et al. (2001) have reported that 52% of mothers in their study were CMV-seropositive, and 22% of uninfected infants exposed to CMV-infected breast milk acquired the virus. The only difference in CMV specific preventive measures taken between these studies was the routine freezing of mother's milk at -20°C in the neonatal unit when an excess of milk was available. This milk was then used at a later date, usually after 72 hours of freezing at -20°C. A study by Friis and Anderson (1982) previously showed that freezing of breast milk at -20°C for more than 72 hours reduces CMV viral titers by 99%. Another showed that overnight freezing of breast milk at -20°C reduced CMV infectivity of milk by 90%, and storage over seven days reduced CMV infectivity by 100% (Stagno et al., 1980). Routine freezing of breast milk at -20°C may reduce transmission of CMV from breast milk of seropositive mothers to their uninfected preterm infants.

CONCLUSIONS

CMV is an agent which causes CID in infants who have acquired the virus in utero, and causes severe systemic disorders due to viral reactivation in patients who are immuno compromised due to HIV-1 infection, organ transplantation, and immunosuppressive chemotherapy. The increase in the popularity of breastfeeding and use of child care arrangements are having a major effect on the epidemiology of cytomegalovirus infections (Stagno et al., 1994). We previously conclude that CMV excreted into milk whey may be more important in vertical infection than that of milk cells infected with CMV for breast-fed infants (Numazaki et al., 2001).

In prospective studies there was a high incidence of CMV infection in preterm infants from seropositive and negative mothers. The most premature infants are at greatest risk of acquiring an early and symptomatic CMV infection. Term infants can be breast-fed when the mother is shedding virus in her milk because of the protection of transplacental maternal antibodies.

Premature infants with low concentration of transplacental antibodies can acquire the disease from the fresh breast milk containing the virus. Freezing breast milk at -20 degrees C for 7 days can inactivate the virus and this may be a protective for the preterm infant until the titer of serum antibody against CMV received by breastfeeding increases.

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