African Journal of Biotechnology Vol. 4 (9), pp.867-872, September 2005
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2005 Academic Journals

Review

Human cytomegalovirus infections in premature infants by breastfeeding

Kei Numazaki

Department of Virology III, National Institute of Infectious Diseases (NIID), Tokyo, Japan E-mail: numazaki@nih.go.jp

Accepted 10 August, 2004

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 at 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 to1990 (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 immuno compromised 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., 1996) also support the data of isolation.

Colostrum and early milk were previously reported to contain abundant I gA and I gM 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 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 administrating 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 post natally 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-α 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 breasted 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℃ 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℃ 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℃ 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℃.A study by Friis and Anderson (1982) previously showed that freezing of breast milk at -20℃ for more than 72 hours reduces CMV viral titers by 99%. Another showed that overnight freezing of breast milk at -20℃ 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℃ 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.

ACKNOWLEDGEMENT

This work was supported by research grants from the Ministry of Education, Science and Culture of Japan.

REFERENCES

Ahlfors K, Ivarsson SA (1985). Cytomegalovirus in breast milk of Swedish milk donor. Scand. J. Infect. Dis. 17: 11-13.

Asanuma H, Numazaki K, Nagata N, Chiba S (1995). Cytokine response and polymerase chain reaction study of peripheral blood mononuclear cells in infants with human cytomegalovirus infection. FEMS Immunol. Med. Microbiol. 12: 153-158.

Asanuma H, Numazaki K, Nagata N, Hotsubo T, Horino K, Chiba S (1996). Role of milk whey in the transmission of human cytomegalovirus infection by breast milk. Microbiol. Immunol. 40: 201-204.

Ballard RA, Drew WL, Hufnagle KG, Riedel PA (1979). Acquired cytomegalovirus in preterm infants. Am. J. Dis. Child. 133: 482-485.

Diosi P, Babusceac L. Nevinglovschi O, Kun-Stoicu G (1967). Cytomegalovirus infection associated with pregnancy. Lancet ii: 1063-1066.

Dworsky M, Stagno S, Pass R, Cassady G, Alford C (1982). Persistence of cytomegalovirus in human milk after storage. J. Paediatrics 101: 440-443.

Dworsky M, Yow M, Stagno S, Pass RF, Alford C (1983). Cytomegalovirus infection of breast milk and transmission in infancy. Paediatrics 72: 295-299.

Fujikawa T, Numazaki K, Asanuma H, Kudo R, Tsutsumi H (2003a). The frequency of human cytomegalovirus-specific T cells during pregnancy determined by intracellular cytokine staining. J. Med. Virol. 71: 527-531.

Fujikawa T, Numazaki K, Asanuma H, Tsutsumi H (2003b). Human cytomegalovirus infection during pregnancy and detection of specific T cells by intracellular cytoine staining. Int . J. Infect. Dis. 7: 215-221.

Friis H, Andersen H (1982). Rate of inactivation of cytomegalovirus in raw banked milk during storage at -20℃ and pasteurisation. Brit . Med. J. 285: 1604-1605.

Goldblum RM, Dill CW, Albrecht TB, Alford ES, Garza C, Goldman AS (1984). Rapid high temperature treatment of human milk. J. Paediatrics 104: 380-385.

Goldman AS, Garza C, Nichols, Goldblum RM (1982). Immunologic factors in human milk during the first year of lactation. J. Pediatrics 100: 563-567.

Grundy JE, Shanley JD, Griffith PD (1987). Is cytomegalovirus interstitial pneumonitis in transplant recipients an immuno-pathological conditions? Lancet ii: 996-999.

Hamprecht K, Maschmann J, Vochem M, Dietz K, Christian P Speer CP, Gerhard J (2001). Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. Lancet 357: 513-518.

Harmsen MC, Swart PJ, de Béthune MP, Pauwels R, de Clercq E, The TH (1995). Antiviral effects of plasma and milk proteins: lactoferrin shows potent activity against both human immunodeficiency virus and human cytomegalovirus replication *in vitro*. J. Infect. Dis. 172: 380-388.

Hayes K, Danks DM, Gibas H, Jack I (1972). Cytomegalovirus in human milk. N. Engl. J. Med. 287: 177-178.

Hirota K, Muraguchi K, Watanabe N, Okumura M, Kozu M, Takahashi K, Machida Y, Funayama Y, Oshima T, Numazaki Y (1992).Prospective study on maternal, intrauterine, and perinatal infections with cytomegalovirus in Japan during 1976-1990. J. Med. Virol. 37: 303-306.

Hotsubo T, Nagata N, Shimada M, Yoshida K, Fujinaga K, Chiba S (1994). Detection of human cytomegalovirus DNA in breast milk by means of polymerase chain reaction. Microbiol. Immunol. 38: 809-811.

Iles R, Lyon A, Ross P, McIntosh N (1996). Infection with *Ureaplasma urealyticum* and *Mycoplasma hominis* and the development of chronic lung disease in preterm infants. Acta Paediatrica 85: 482-484.

Kinoshita K, Hino S, Amagasaki T, Ikeda S, Yamada Y, Suzuyama J, Momita S, Toriya K, Kamihira S, Ichimura M (1984). Demonstration of adult T-cell leukemia virus antigen in milk from three sero-positive mothers. Jpn. J. Cancer Res. (Gann) 175: 103-105.

Kumar ML, Nankervis GA, Cooper AR, Gold E (1984). Postnatally acquired cytomegalovirus infections in infants of CMV-excreting mothers. J. Pediatr. 104:669-673.

Lawrence RA (1999). Storage of human milk and the influence of procedures on immunological components of human milk. Acta Paediatrica 88 (Suppl):14-18.

Minamishima I, Ueda K, Minematsu T, Minamishima Y, Umemoto M, Take H, Kuraya K (1994). Role of breast milk in acquisition of cytomegalovirus infection. Microbiol. Immunol. 38: 549-552.

Nishimura N, Kimura H, Yabuta Y, Tanaka N, Ito Y, Ishikawa K, Suzuki C, Morishima T (1999). Prevalence of maternal cytomegalovirus (CMV) antibody and detection of CMV DNA in amniotic fluid. Microbiol. Immunol . 43: 781-784.

Numazaki K, Chiba S, Kogawa K, Umetsu M, Motoya H, Nakao T (1986). Chronic respiratory disease in premature infants caused by *Chlamydia trachomatis*. J. Clin. Pathol. 39: 84-88.

Numazaki K, Asanuma H, Nagata N, Chiba S (1994). Analysis of human cytomegalovirus-infected peripheral blood mononuclear cells from infants with liver dysfunction by flow cytometry and the polymerase chain reaction. J. Leukocyte Biol. 56: 187-191.

Numazaki K, Asanuma H, Hotsubo T, Chiba S (1996). Anti-human cytomegalovirus effects of breast milk. J. Infect. Dis. 174:444.

Numazaki K, Chiba S (1996). PCR detection cytomegalovirus DNA in serum as test for congenital cytomegalovirus infections. J. Clin. Microbiol. 34: 1871-1872.

Numazaki K (1997). Human cytomegalovirus infection of breast milk. FEMS Immunol. Med. Microbiol. 18: 91-98.

Numazaki K, Asanuma H, Chiba S (1997). Relationship between cytokines and human cytomegalovirus infection of breast milk. J. Infect. Chemotherapy 3: 58-61.

Numazaki K, Chiba S (1997). Current aspects of diagnosis and treatment of cytomegalovirus infections in infants. Clin. Diagn. Virol. 8: 169-181.

Numazaki K, Asanuma H, Ikehata M, Chiba S (1998). Detection of cytokines and cytomegalovirus DNA in serum as test for congenital infection. Early Human Dev. 52: 43-48.

Numazaki K, Fujikawa T, Chiba S (2000). Relationship between seropositivity of husbands and primary cytomegalovirus infection during pregnancy. J. Infect. Chemotherapy. 6: 104-106.

Numazaki K, Chiba S, Asanuma H (2001). Transmission of cytomegalovirus. Lancet 357: 1799-1800.

Numazaki K, Fujikawa T (2002). Prevalence of serum antibodies to cytomegalovirus in pregnant women in Sapporo, Japan. Int. J. Infect. Dis. 6: 147-148.

Numazaki K, Fujikawa T, Asanuma H (2002). Immunological evaluation and clinical aspects of children with congenital cytomegalovirus infection. Congenital Anomalies 42: 181-186.

Numazaki K, Fujikawa T (2004). Chronological changes of incidence and prognosis of children with asymptomatic congenital cytomegalovirus infection in Sapporo, Japan. BMC Infectious Diseases, 4: 22.

Pierce MR, Bancalari E (1995). The role of inflammation in the pathogenesis of bronchopulmonary dysplasia. Paediatrics Pulmonol. 19: 371-378.

Prosch S, Lienicke U, Priemer C, Flunker G, Seidel WF, Kruger DH,

Wauer RR (2002). Human adenovirus and human cytomegalovirus infections in preterm newborns: no association with bronchopulmonary dysplasia. Paediatrics Res . 52: 219-224.
Sawyer MH, Edwards DK, Spector SA (1987). Cytomegalovirus infection and bronchopulmonary dysplasia in preterm infants. Am. J. Dis. Child. 141: 303-305.
Skansén-Saphir U, Lindfors A, Andersson U (1993) Cytokine production in mononuclear cells of human milk studied at the single-cell level. Paediatrics Res. 34: 213-216.
Snydman DR, Werner BG, Meissner HC, Cheeseman SH, Schwab J, Bednarek F, Kennedy JL Jr, Herschel M, Magno A, Levin MJ (1995). Use of cytomegalovirus immunoglobulin in multiply transfused premature neonates. Paediatrics Infect. Dis. J. 14: 34-40.
Speer CHP, Gahr M, Pabst MJ (1986). Phagocytosis-associated oxidatove metabolism in human milk macrophages. Acta Paediatrics Scand. 75: 444-451.
Stagno S, Reynolds DW, Pass RF, Alford CA (1980). Breast milk and the risk of cytomegalovirus infection. N. Engl. J. Med. 302: 1073-1076.
Stagno S, Cloud GA (1994). Working parents: the impact of day care and breast-feeding on cytomegalovirus infections in offspring. Proc. Natl. Acad. Sci. USA 91: 2384-2389.
Van de Perre P, Simonon A, Hitimana D-G, Dabis F, Mstellati P, Mukamabano B, Butera JB, Van Goethem C, Karita E, Lepage P: (1993). Infective and anti-infective properties of breast milk from HIV-1-infected women. Lancet 341: 914-918.
Vochem M, Hamprecht K, Jahn G, Speer CP (1998). Transmission of cytomegalovirus to preterm infants through breast milk. Paediatrics. Infect. Dis. J. 17: 53-58.
Wang EEL, Ohlsson A, Kellner JD (1995). Association of *Ureaplasma urealyticum* colonization with chronic lung disease of prematurity: results of a metaanalysis. J. Paediatrics 127: 640-644.
Welsh JK, Arsenakis M, Coelen RJ, May JT (1979). Effect of antiviral lipids, heat and freezing on the activity of viruses in human milk. J. Infect. Dis. 140: 322-326.
Wright KC, Feeney AM (1998). The bacteriological screening of donated human milk: laboratory experience of British Paediatrics Association's published guidelines. J. Infect. 36: 23-27.
Yeager AS, Palumbo PE, Malachowski N, Ariagno RL, Stevenson DK (1983). Sequelae of maternally derived cytomegalovirus infections in preterm infants. J. Pediatr. 102: 918-922 .

Journal of Medical Virology 76:356–360 (2005)

Human Cytomegalovirus Genetic Variability in Strains Isolated From Japanese Children During 1983–2003

Kaori Tanaka, Kei Numazaki, and Hiroyuki Tsutsumi*

Department of Pediatrics, Sapporo Medical University School of Medicine, Sapporo, Japan

The genetic variability of 74 human cytomegalovirus (HCMV) clinical isolates from 60 Japanese infants and children during 1983–2003 was investigated, and the relevance to their clinical course was studied. The patients consisted of 10 asymptomatic congenitally infected babies, 45 infected perinatally or postnatally resulting in HCMV mononucleosis/hepatitis and 5 immunocompromised hosts. The hypervariable region of the HCMV genome, that is the *a* sequence and UL144 region was analyzed using the polymerase chain reaction (PCR) and unrooted phylogenetic trees. HCMV glycoprotein B (gB) polymorphism was also studied. Unrooted phylogenetic trees of *a* sequence and UL144 allowed the isolates to be grouped to 5 and 3 clades, respectively. Three gB genotypes were also determined. However, there was no correlation between specific genotypes of these three genes and clinical forms, except for congenital infection which fell into one of three clades of the *UL144* gene. In addition, the variability of the three genes had no correlation with each other. This implies that study of a single gene is insufficient for investigating the molecular epidemiology of HCMV. This study provides basic data on the genetic variability of HCMV in an Asian population and should help to determine the strains for vaccine candidates. ***J. Med. Virol. 76:356–360, 2005.*** © 2005 Wiley-Liss, Inc.

KEY WORDS: molecular epidemiology; congenital infection; human cytomegalovirus (HCMV); HCMV glycoprotein B; HCMV *a* sequence; HCMV *UL144*

INTRODUCTION

Human cytomegalovirus (HCMV) is a herpes virus and contains approximately 240 Kb DNA and over 200 open reading frames (ORFs) [Bale et al., 2001; Walker et al., 2001; Schleiss, 2003]. Although HCMV infection is usually asymptomatic, it is the most common cause of intrauterine infection throughout the world. Intrauterine infection with HCMV carries the risk of a possible severe neurologic outcome in infants [Fowler et al., 1992; Murph et al., 1998; Demmler, 1999; Noyola et al., 2000]. HCMV antibodies are found in about 40%–60% of adults in Western countries [Clarke et al., 1996], and about 80%–90% of Japanese adults [Tanaka et al., 1998]. However, in recent years the HCMV infection rate in adult women has been decreasing, raising concerns of an increase in congenital infections secondary to primary maternal infection during pregnancy. Furthermore, current studies suggest that HCMV seropositive pregnant women can be re-infected with different HCMV strains and those re-infections may lead to intrauterine transmission and symptomatic congenital infections [Adler et al., 1995; Boppana et al., 1999, 2001].

The relationship between HCMV genetic variability and disease outcome has been the focus of many studies because this is expected to provide the basis for preventing infection or improving disease prognosis. Molecular epidemiological studies revealed the hypervariability of the glycoprotein B (*gB*) gene among HCMV; however, the correlation between gB genotypes and clinical symptoms remains unclear [Lukacsi et al., 2001; Humar et al., 2003; Rasmussen et al., 2003]. In addition, HCMV congenital infections have seldom been considered from the point of view of viral genetic heterogeneity [Bale et al., 2000].

HCMV is composed of unique long (L) and short (S) sequences containing terminal segments with repeating elements. The HCMV *a* sequence is located in the joining region between L and S sequences [Zaia et al., 1990; Boger et al., 2002]. Recently, the epidemiological relationships between variation of the *a* sequence gene and HCMV clinical isolates were reported [Zaia et al., 1990; Bale et al., 1996, 2001; Walker et al., 2001].

*Correspondence to: Hiroyuki Tsutsumi, MD, PhD, Department of Pediatrics, Sapporo Medical University School of Medicine, S-1, W-16, Chuo-ku, Sapporo 060-8543, Japan.
E-mail: tsutsumi@sapmed.ac.jp

Accepted 7 December 2004

DOI 10.1002/jmv.20366

Published online in Wiley InterScience (www.interscience.wiley.com)

Hypervariability of *UL144* gene sequences has also been reported [Lurain et al., 1999]. UL144 is one of the ORFs and encodes a homolog of the herpes simplex virus entry mediator [Benedict et al., 1999; Bale et al., 2001]. UL144 ORF can be categorized into three major groups, however no apparent correlation between UL144 variants and HCMV pathogenesis has been identified [Bale et al., 2001].

There have been no similar molecular epidemiological studies on HCMV isolates from an Asian population except for a few studies on gB [Shiu et al., 1994; Numazaki et al., 2000]. HCMV clinical strains isolated from Japanese infants and children during the last two decades were studied. Genetic variability in the *a* sequence, UL144 region, and *gB* genes, and their association with clinical course was investigated. In addition, the findings were compared with those from Western countries.

MATERIALS AND METHODS

Virus Strains

Seventy-four HCMV strains were studied. These wereisolated during 1983–2003 from the urine of 60 Japanese infants and children in Sapporo, Japan. Of these, 10 newborn babies were confirmed to have asymptomatic congenital HCMV infection by routine screening of urine for HCMV excretion. Forty-five patients (1 month–5 years 4 months; mean, 11 months), all immunologically normal, had a HCMV mononucleosis or hepatitis by perinatal or horizontal infection. Five immunocompromised hosts (9 years 10 months–23 years 1 month; mean, 15 years 11 months) who were receiving anti-cancer or immunosupressive agents were included as HCMV reactivation. Of the 74 strains, 24 were collected from 10 children, that is, 2–3 strains were isolated serially from the same patients. The interval between each isolation ranged from 1 month to 1 year. The laboratory strain AD169 was also included. Samples of urine were inoculated onto MRC-5 cells for HCMV isolation. Virus isolates were stored at −80°C until further examination.

DNA Isolation

Virus isolates were cultured in MRC-5 cells in 24-well semi-microplates containing 1 ml of culture medium (Eagle's MEM with 2% fetal calf serum) per well. When an extensive cytopathic effect was present, the cells were washed once and mixed viral genomic and cellular DNA was extracted using QlAmp DNA Minikit (QIAGEN, Inc., Valencia, CA). Isolates were passaged less than four times prior to DNA extraction. Of 74 stocked-virus strains, 48 could be re-isolated with tissue culture; however, the remaining 26 strains could not, and DNA was extracted from stored-virus fluid directly.

PCR Amplification

Three regions of the HCMV genome, that is, the *a* sequence, UL144, and gB, were analyzed using polymerase chain reaction (PCR). Forward primer for the *a* sequence region was our original (TTCC CCGGGGAATCAAACAG), and reverse primer was described by Zaia et al. [1990] (TTTTTAGCGGGGGGGTGAAA). The UL144 region was amplified using the primer pair described by Lurain et al. [1999] (forward: TCGTATTACAAACCGCGGAGAGGAT; reverse: ACTCAGAC ACGGTTCCGTAA).

After denaturing at 94°C for 5 min and cooled to 80°C, the PCR mixture was seeded with thermostable Taq polymerase (Promega, Madison, WI). Forty cycles of amplification were carried out with a DNA thermal cycler (PE-ABI, Foster city, CA). Each cycle consisted of warming at 95°C for 40 sec, 55°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. Amplicons were visualized on 2% agarose gels.

gB genotypes were determined by PCR-RFLP analysis using enzymes Hinf I and Rsa I as described by Chou and Dennison [1991].

DNA Sequencing

The concentrated PCR products were sequenced directly with the BigDye Terminator Cycle Sequencing Kit (PE-ABI). The sequencing products were analyzed on an ABI PRISM 3100 Genetic Analyzer (PE-ABI). Consensus nucleotide sequences for the *a* sequence and UL144 were determined by visual inspection of forward and reverse strands.

Phylogenetic Analysis

Sequence alignments were accomplished using web based Clustal W alignment programs. Unrooted phylogenetic trees were constructed for the *a* sequence and UL144 DNA sequence data using the above website. They were visualized and edited using TREE VIEW.

RESULTS

a Sequence Variability

At first, attempts were made to amplify the *a* sequence gene using the primer pair described by Zaia et al. [1990]; however, only 9 of the 74 (12%) strains (7 of the 48 re-isolated strains and 2 of the 26 stocked-virus fluid) could be amplified. Therefore, the forward primer was changed to our original one. As a result, the *a* sequence of 60 of the 74 (81%) strains (39 re-isolated strains and 21 stocked-virus fluid) could be amplified. The PCR products ranged rather broadly in length from 162bp to 238bp.

PCR products were sequenced directly. Analysis of the nucleotide sequences of the region of 60 strains revealed differences from 0% to 43% between strains. Phylogenetic analysis was conducted with 48 strains except for 12 strains which were isolated from the same subjects and had identical sequences (Fig. 1). In the unrooted tree, there were five major groups (A, B1, B2, C1, and C2) approximately (Fig. 1).

Each cluster consisted of 19, 8, 8, 9, and 4 strains, respectively. There was 0%–27% nucleotide differences

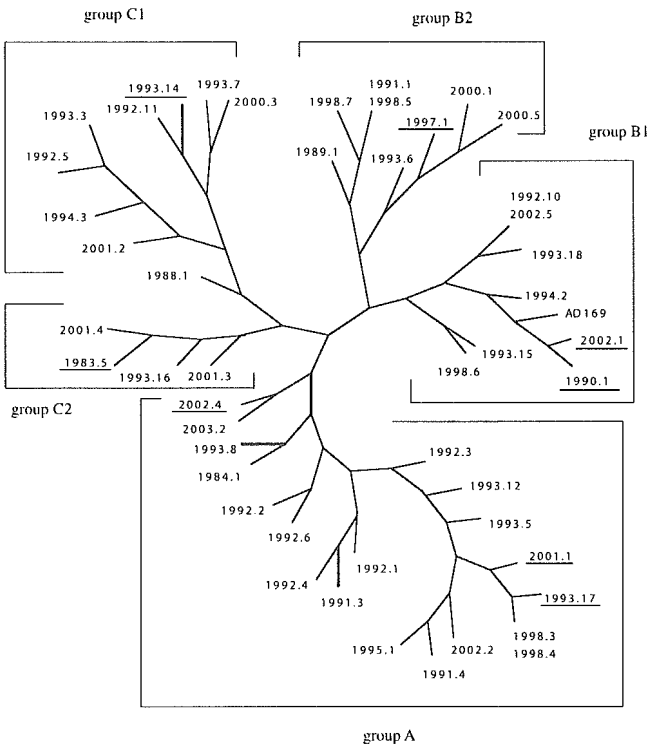

Fig. 1. Unrooted phylogenetic dendrogram showing the relationship of 48 human cytomegalovirus (HCMV) strains with respect to *a* sequence polymorphisms. AD169 strain is included. Numbers with underlines indicate isolates from congenital infection.

among strains located in the same group. They contained strains which were isolated from subjects with variable clinical symptoms. Each cluster includes 1–3 isolates from infants with a congenital infection. The dates of isolation of strains belonging to each group were 1984–2002, 1990–2002, 1989–2000, 1988–2001, and 1983–2001 in groups A, B1, B2, C1, and C2, respectively. Serial isolates from the same patients possessed identical sequences for *a* sequence regions.

UL144 Variability

Sixty-three (85%) strains (all 48 re-isolated strains and 15 stocked-virus fluid) yielded *UL144* gene PCR products which had 737-bp length. The HCMV laboratory strain, that is AD169 lacks 19 ORFs including UL144, thus AD169 did not yield amplicons [Cha et al., 1996; Lurain et al., 1999; Bale et al., 2001].

Unrooted phylogenetic trees were edited using 52 field isolates except for 11 isolates which had identical sequences from the same subjects (Fig. 2). This schema segregated 52 strains into three major groups. Phylogenetic trees were also made with our 52 isolates and the strains described in Lurain et al. [1999]. We confirmed that the group designations of our strains (1–3) conformed to the scheme proposed by them (data not shown).

Each group consisted of 21, 7, and 24 strains, respectively. Analysis of the nucleotide sequence of the region of 52 strains revealed differences from 0% to 17%

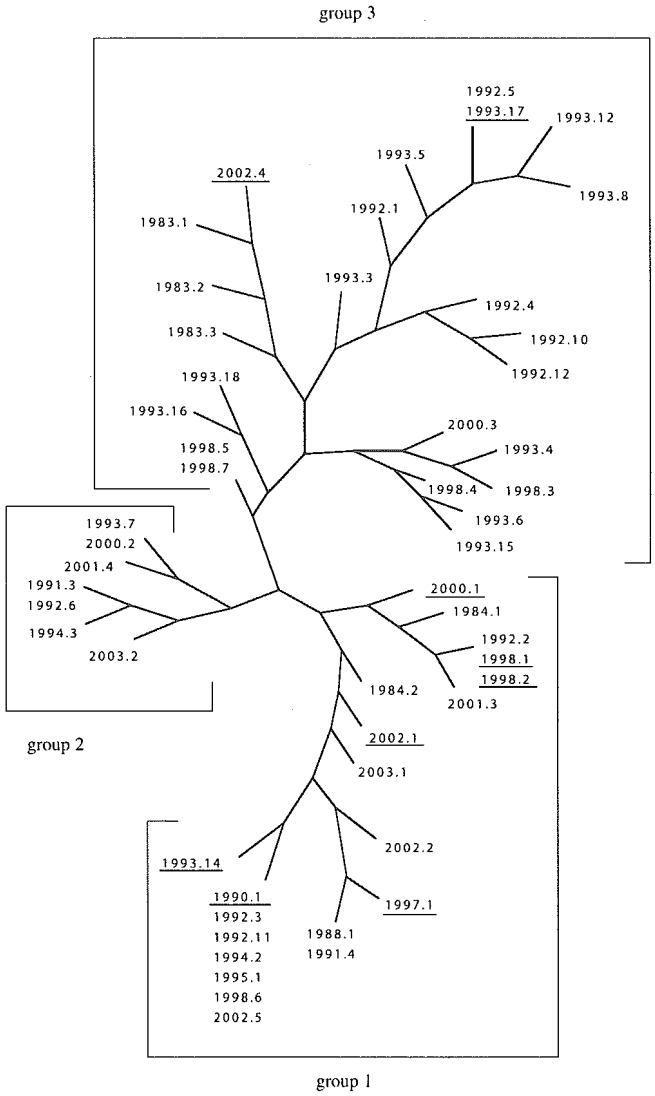

Fig. 2. Unrooted phylogenetic dendrogram showing the relationship of 52 HCMV strains with respect to *UL144* gene variability. Numbers with underlines indicate isolates from congenital infection. Group designations conform to the scheme proposed by Lurain et al. [1999].

between strains. On the other hand, there were only 0%–5% nucleotide differences among strains located in the same group.

The dates of isolations of strains belonging to each group extended over a long time, that is 1982–2002, 1991–2003, and 1983–2002 in groups 1, 2, and 3,

TABLE I. Distribution of Human Cytomegalovirus (HCMV) Glycoprotein B (gB) Genotype and Type of Infection

Group	1	2	3	4
Congenital infection	6	2	1	0
Perinatal/horizontal infection	26	0	15	0
Immunocompromised host	2	0	1	0
Total	34	2	17	0

Fifty-three strains were analyzed.

respectively. In group 1, strains from patients with congenital infections accounted for 33.3% (7/21), however, in groups 2 and 3, they accounted for just 0% (0/7) and 8.3% (2/24), respectively; this difference was statistically significant (χ^2 test, $P < 0.05$).

Nine children serially secreted viruses with identical UL144 sequences. One child secreted different strains at 1 year 7 months and 2 years 2 months old; in these the *UL144* gene differed at 25 of 624 nucleotide positions. These two strains, that is, 1984.1 and 1984.2, were categorized to different branches within group 1 (Fig. 2).

gB Variability

The *gB* gene of 67 of 74 (90%) strains (all 48 re-isolated strains and 19 stocked-virus fluid) could be amplified and analyzed by RFLP assay using enzymes, Hinf I and Rsa I. Excepting the 14 strains isolated from the same subjects, 34, 2, and 17 were identified as gB group 1, 2, and 3, respectively (Table I). None of our strains were classified as group 4. There was no obvious correlation between a certain gB genotype and the type of disease. AD169 was identified as group 2.

Linkage of *a* Sequence, UL144 Polymorphism, and gB

Strains were compared according to their unrooted phylogenetic dendrogram of *a* sequence, UL144, and gB genotypes. No apparent linkage of polymorphisms or genotypes was observed. Group A was the dominant genotype in *a* sequence (39.6% of all isolates), group 3 in UL144 (46.2% of all isolates), and group 1 in gB (68.7% of all isolates). But no relationships were apparent between the dominant genotypes. For example, strains belonging to UL144 group 3 were dispersed to five groups of *a* sequence and two groups of gB in almost equal ratios (data not shown).

DISCUSSION

This is the first report on HCMV *a* sequence and UL144 molecular epidemiology from an Asian country. In the unrooted tree of the *a* sequence region, 48 isolates could be classified into five groups. Each group contained 4–19 strains. However, no apparent linkage of clusters and clinical diseases was observed. The isolation date of strains within each group were varied widely, thus there was no temporal variation of field strains. Serial isolates from the same patients possessed identical sequences in the *a* sequence region. This suggests that the HCMV does not easily change within subjects even at the locus of the hypervariable *a* sequence gene.

Bale et al. [2001] analyzed the genetic variability of *a* sequences of HCMV strains from healthy children in a day care center and from subjects with a congenital infection. They also divided strains into five groups which primarily corresponded to nosocomial infection within the day care center, however any correlation between our five groups and theirs could not be tested because sequence data on their HCMV strains was unavailable.

In the unrooted tree of the UL144 region, our 63 strains could be clearly classified into three major groups (1–3). Lurain et al. [1999] analyzed UL144 genotypes of clinical HCMV isolates from immunocompromised patients who had had organ transplantation or HIV infection, while Bale et al. [2001] analyzed HCMV from healthy children and neonates. All studies, including in the present study, divided HCMV strains into three major groups with similar designations (1–3). The patient's clinical details in these three studies were different, and there was considerable geographical and temporal variation. However, all showed that groups 1 and 3 were large and group 2 was the smallest. Therefore, the HCMV genetic distribution pattern was similar irrespective of the geographical or temporal variation, and of the genetic background of the host, in terms of UL144 genetic variability.

In our study of the *UL144* gene, almost all strains causing congenital infection were located in group 1, in contrast Bale et al. [2001] found that strains causing congenital infections belonged mainly to group 3. This difference cannot be resolved given the small number of cases of congenital infection studied and a much larger study of HCMV strains is required to determine the correlation between congenital infection and UL144 genotype. If particular strain genotypes do infect fetuses more readily, such knowledge would be helpful in predicting congenital infections and developing preventative measures.

Lurain et al. [1999] reported that the UL144 sequence was maintained in all isolates from the same patient, but Bale et al. [2001] found that serial strains from two of four children studied displayed differences. We found that the UL144 sequence of serial strains from 9 of 10 children was identical. But in one child, the isolate at 1 year 7 months differed genetically from that at 2 years 2 months. In the unrooted phylogenetic dendrogram, these two strains fall into different branches, although within the same group, which implies repeated infection with distinct HCMV strains. However, the sequences of the *a* sequence region and gB genotypes could not differentiate these two strains, emphasizing the need for plural analysis for differentiation of CMV field strains.

gB is a major component of the virion envelope and is transported to the plasma membrane of infected cells [Meyer-Konig et al., 1998a; Plotkin, 1999]. Chou and Dennison [1991] classified HCMV strains into four variant groups by PCR-RFLP. The hypervariability of the gB region is well established and the correlation between gB and pathogenicity has been examined without any obvious relationship being found [Meyer-Konig et al., 1998b; Lurain et al., 1999; Lukacsi et al., 2001; Humar et al., 2003]. No correlation was found between gB genotype and the type of disease, but the numbers of subjects analyzed in the current study were small. In addition, we do not know the distribution of gB genotypes among healthy persons living in the Sapporo region.

In this study, the gB group 4 isolate was very uncommon (0 of all isolates), which phenomenon has now been observed among HCMV strains isolated in USA and Germany [Meyer-Konig et al., 1998; Xanthakos and Schleiss, 2003]. Therefore, this may reflect the recent epidemiologic appearance of gB 4 strains, or differences in infectivity or virulence. Further investigations will be needed to elucidate these questions.

In the present study, three genes of HCMV field strains were investigated and great variability in each gene was observed, however there was no correlation between variations. This implies that study of a single gene is inadequate for investigating the molecular epidemiology of HCMV. The infection and pathogenesis of an individual HCMV strain may be defined by the combination of multiple variant genes that it encodes. The genomic variability of HCMV is important to efforts to develop a HCMV vaccine [Plotkin, 1999]. This study provides some basic data on the genetic variability of HCMV in an Asian population and should help to determine the optimal strains for vaccine development.

REFERENCES

Adler SP, Starr SE, Plotkin SA, Hempfling SH, Buis J, Manning ML, Best AM. 1995. Immunity induced by primary human cytomegalovirus infection protects against secondary infection among women of childbearing age. J Infect Dis 171:26–32.

Bale JF, Jr., Petheram SJ, Souza IE, Murph JR. 1996. Cytomegalovirus reinfection in young children. J Pediatr 128:347–352.

Bale JF, Jr., Murph JR, Demmler GJ, Dawson J, Miller JE, Petheram SJ. 2000. Intrauterine cytomegalovirus infection and glycoprotein B. genotypes. J Infect Dis 182:933–936.

Bale JF, Jr., Petheram SJ, Robertson M, Murph JR, Demmler G. 2001. Human cytomegalovirus a sequence and UL144 variability in strains from infected children. J Med Virol 65:90–96.

Benedict CA, Butrovich KD, Lurain NS, Corbeil J, Rooney I, Schneider P, Tschopp J, Ware CF. 1999. A novel viral TNF receptor superfamily member in virulent strains of human cytomegalovirus. J Immunol 162:6967–6970.

Boger RA, Willoughby RE, Pass RF, Zong JC, Jang WJ, Alcendor D, Hayward GS. 2002. Polymorphisms of the cytomegalovirus (CMV)—encoded tumor necrosis factor—α- and β-chemokine receptors in congenital CMV disease. J Infect Dis 186:1057–1064.

Boppana SB, Fowler KB, Britt WJ, Stagno S, Pass RF. 1999. Symptomatic congenital cytomegalovirus infection in infants born to mothers with preexisting immunity to cytomegalovirus. Pediatrics 104:55–60.

Boppana SB, Rivera LB, Fowler KB, Mach M, Britt WJ. 2001. Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. N Eng J Med 344:1366–1371.

Cha TA, Tom E, Kemble GW, Duke GM, Mocarski ES, Spaete RR. 1996. Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains. J virol 70:78–83.

Chou S, Dennison KM. 1991. Analysis of interstrain in cytomegalovirus glycoprotein B sequences encoding neutralization-related epitopes. J Infect Dis 163:1229–1234.

Clarke LM, Duerr A, Feldman J, Sierra MF, Daidone BJ, Landesman SH. 1996. Factors associated with cytomegalovirus infection among human immunodeficiency virus type 1-seronegative and -seropositive women from an urban minority community. J Infect Dis 173: 77–82.

Demmler GJ. 1999. Congenital cytomegalovirus infection and disease. Semin Pediatr Infect Dis 10:195–200.

Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ, Alford CA. 1992. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Eng J Med 326:663–667.

Humar A, Kumar D, Gilbert C, Boivin G. 2003. Cytomegalovirus (CMV) glycoprotein B genotypes and response to antiviral therapy, in solid-organ-transplant recipients with CMV disease. J Infect Dis 188:581–584.

Lukacsi A, Tarodi B, Endreffy E, Babinszki A, Pal A, Pusztai R. 2001. Human cytomegalovirus gB genotypes 1 is dominant in congenital infections in south Hungary. J Med Virol 65:537–542.

Lurain NS, Kapell K, Huang DD, Short JA, Paintsil J, Winkfield E, Benedict CA, Ware CF, Bremer JW. 1999. Human cytomegalovirus UL144 open reading frame: Sequence hypervariability in low-passage clinical isolates. J Virol 73:10040–10050.

Meyer-Konig U, Vogelberg C, Bongarts A, Kampa D, Delbruk R, Wolff-Vorbeck G, Kirste G, Haberland M, Hufert FT, Laer D. 1998a. Glycoprotein B genotype correlates with cell tropism in vivo of human cytomegalovirus infection. J Med Virol 54:75–81.

Meyler-Konig U, Haberland M, Laer D, Haller O, Hufert FT. 1998b. Intragenic variability of human cytomegalovirus glycoprotein B in clinical strains. J Infect Dis 177:1162–1169.

Murph JR, Souza IE, Dawson JD, Benson P, Petheram SJ, Pfab D, Gregg A, O'Neill ME, Zimmerman B, Bale JF, Jr. 1998. Epidemiology of congenital cytomegalovirus infection: Maternal risk factors and molecular analysis of cytomegalovirus strains. Am J Epidemiol 147:940–947.

Noyola DE, Demmler GJ, Williamson D, Griesser C, Sellers S, Llorente A, Littman T, Williams S, Jarrett L, Yow MD, The congenital CMV longitudinal study group. 2000. Cytomegalovirus urinary excretion and long term outcome in children with congenital cytomegalovirus infection. Pediatr Infect Dis J 19:505–510.

Numazaki K, Ikehata M, Chiba S. 2000. Subtyping of cytomegalovirus strains obtained from immunocompetent children. In Vivo 14:745–746.

Plotkin SA. 1999. Vaccination against cytomegalovirus, the changeling demon. Pediatr Infect Dis J 18:313–326.

Rasmussen L, Geissler A, Winters M. 2003. Inter- and intragenic variations complicate the molecular epidemiology of human cytomegalovirus. J Infect Dis 187:809–819.

Schleiss MR. 2003. Vertically transmitted herpesvirus infections. Herpes 10:4–11.

Shiu SY, Chan KM, Lo SK, Ip KW, Yuen KY, Health RB. 1994. Sequence variation of the amino-terminal antigenic domains of glycoprotein B of human cytomegalovirus strains isolated from Chinese patients. Arch Virol 137:133–138.

Tanaka N, Kimura H, Morishima T. 1998. Progress in the management of cytomegalovirus (CMV) infection. Jpn J Clin Med 56:167–172 (in Japanese).

Walker A, Petheram SJ, Ballard L, Murph JR, Demmler GJ, Bale JF, Jr. 2001. Characterization of human cytomegalovirus strains by analysis of short tandem repeat polymorphisms. J Clin Microbiol 39:2219–2226.

Xanthakos SA, Schleiss MR. 2003. Glycoprotein B genotyping of cytomegalovirus strais isolated in a pediatric population. Pediatr Infect Dis J 22:462–463.

Zaia JA, Howkins GG, Churchill MA, Blackshere AM, Pande H, Adler SP, Schmidt GM, Forman SJ. 1990. Comparative analysis of human cytomegalovirus a-sequence in multiple clinical isolates by using polymerase chain reaction and restriction fragment length polymorphism assays. J Clin Microbiol 28:2602–2607.

Respiratory tract infections due to *Chlamydia trachomatis* in early neonatal period

Kei Numazaki[1)], Hideomi Asanuma[1,2)] and Yuichi Niida[1,2)]

[1)] Department of Pediatrics, Sapporo Medical University School of Medicine, Sapporo, Japan
[2)] Hokkaido Children's Hospital and Medical Center Otaru, Otaru, Japan

Introduction

Chlamydia trachomatis has been recognized as a pathogen of nongonococcal urethritis (NGU), salpingitis, endocervicitis, pelvic inflammatory disease (PID), inclusion conjunctivitis of neonates, follicular conjunctivitis of adults, infantile pneumonia and associated conditions[1)]. Genital or ophthalmic chlamydial infections are still recognized as a major public health problem throughout the world including Japan.

Pneumonia due to *C. trachomatis* is a disease limited for the most part to infants less than 6 months of age. It has been suggested that *C. trachomatis* infection in pregnant women may be related to premature labor and to perinatal death. Although transmission of the organism from mothers to their infants generally occurs at the time of delivery with passage of the infant through the infected cervix, the possibility of intrauterine infection at late pregnancy has been reported[2,3)].

Reported below are the cases of *C. trachomatis* respiratory tract infections in early neonatal period with the possibility of intrauterine infection.

Materials and methods

1. Patients

Case 1 was a female born by vaginal delivery after 39 weeks' gestation with a birth weight 2,605 g ; Case 2 was also a female born after 40 weeks' gestation by Caesarean section because of fetal distress with a birth weight of 3,025 g. No asphyxia was found at birth and there was no episode of premature rupture of membrane (PROM). Tachypnea or cyanosis developed days 3 and 13 in Case 1 and 2 respectively. Case 3 was a male born by vaginal delivery after 37 weeks' gestation with a birth weight of 2,982 g ; Case 4 was a female born after 33 weeks' gestation also by vaginal delivery

with a birth weight of 2,265 g. There was an episode of meconium-stained amniotic fluid with Case 3. Chorioamnionitis was found in the placenta of Case 4. Wheeze and tachypnea developed within one day after delivery. Case 5 was a female born after 37 weeks' gestation also by vaginal delivery with a birth weight of 3,714 g. Tachypnea and cyanosis developed from day 1 in Case 5.

Chest radiographs of Cases 1, 2, 3 and 4 on admission showed streaky shadows and reticulogranular infiltrates over whole lung without hyperinflation. That of Case 5 on admission showed streaky shadow over the whole lung without hyperinflation. Blood examination of Cases 1 and 2 revealed increased C-reactive protein (13.1 and 6.5 mg/dL) and hyperleukocytosis (18,700 and 31,200/μL) without eosinophilia.

Blood examination of Cases 3 and 4 revealed normal or slightly increased C-reactive protein (0.10 and 0.00 mg/dL) and hyperleukocytosis (38,500/μL) in Case 4. Bacterial cultures from throat, urine, blood, feces and cerebrospinal fluid were all negative. Human cytomegalovirus (CMV) was isolated from urine of Case 5. Serum IgG and IgM antibodies against CMV were detected from maternal and infantile sera. Blood examination revealed increased C-reactive protein (2.90 mg/dL) and hyperleukocytosis (20,800/μL) without eosinophilia.

2. Microbiological Methods

Nasopharyngeal specimens were collected from the five infants within 2 weeks after birth for antigen detection and the polymerase chain reaction (PCR) study. Endocervical swabs of mothers during pregnancy were also obtained. The PCR assay to amplify *Omp* 1 gene and restriction fragment length polymorphism (RFLP) analysis were used to detect and distinguish serotypes from genotypes of *C. trachomatis*[4,5]. At the first step, 1.4 kbp DNA fragment that is larger than a full length of the *Omp* 1 gene was amplified. At the second step for nested PCR, 1.2 kbp DNA fragment, a full length of *Omp* 1 gene was amplified. Genotyping was performed by *Hinf* I, *Hind* III and *Hha* I restriction analysis of amplified *Omp* 1.

A commercially available EIA kit was also used to detect genus-specific chlamydial antigens[6,7]. Maternal serum, infantile serum and cord blood samples were obtained for standard microimmunofluorescence (MIF) assay to detect IgG and IgM antibodies against *C. trachomatis.*

Table 1 Clinical and laboratory findings of five cases

Case	1	2	3	4	5
Sex	F	F	M	F	F
Gestational age (weeks)	39	40	37	33	37
Birth weight (g)	2,605	3,025	2,982	2,265	3,714
Mode of delivery	Vaginal	Caesarean	Vaginal	Vaginal	Vaginal
Apgar score at 1 min after birth	9	8	7	8	7
Age at onset (days after birth)	3	13	1	0	1
Symptoms at onset	Tachypnea	Cyanosis	Wheeze	Tachypnea	Tachypnea
Nasopharyngeal chlamydial antigen	+	+	+	+	+
Nasopharyngeal chlamydial DNA	+	+	+	+	+
Specific serum IgM antibodies					
Cord blood	1 : 128	< 1 : 16	< 1 : 16	1 : 32	1 : 32
21 days after birth	1 : 64	1 : 32	1 : 32	1 : 16	< 1 : 16

Table 2 Maternal microbiological findings during pregnancy

Mother of Case	1	2	3	4	5
Premature rupture of membranes	no	no	no	no	no
Findings of placenta	unknown	unknown	unknown	Chorio-amnionitis	normal
Presence of endocervical chlamydial antigen	no	no	no	no	no
Serum antibodies to *C. trachomatis* (20 weeks' gestation)					
IgG	1 : 256	not tested	not tested	1 : 256	1 : 256
IgM	< 1 : 16	not tested	not tested	1 : 64	< 1 : 16

Results

Diagnosis of *C. trachomatis* infection was made by antigen detection or PCR assay in nasopharyngeal swabs and by the presence of specific serum IgM antibodies by MIF. The serovar that we identified from nasopharyngeal swabs of these infants was type E only. Treatment with ampicillin and amikacin was initiated without success. Respiratory tract symptoms and radiological appearance improved gradually after oral clarithromycin administration (15 mg/kg per day) for total of 14 to 28 days respectively. Clinical and laboratory findings of the five cases are summarized in Table 1. We also examined mothers of the five infants for *C. trachomatis* infections. These mothers had negative chlamydial EIAs during pregnancy (Table 2).

Discussion

C. trachomatis infections during pregnancy may cause a variety of perinatal complications. Low-birth-weight infants and PROM occurred more frequently in women infected with *C. trachomatis*. The fact that neonates having the symptoms of chronic lung diseases also manifested elevated serum IgM levels suggested that these respiratory-tract disorders arose from intrauterine infections during late pregnancy[8]. *C. trachomatis* can lead to chorioamniotic infection. Chorioamnionitis is a frequent finding in prematurity and respiratory insufficiency in premature babies and may be attributable to intrauterine infection.

We evaluated the significance of detection of serum antibodies to *C. trachomatis* by ELISA at different time of pregnancy for early diagnosis of perinatal complications[9]. The incidence of perinatal complications was significantly higher in IgG and IgA antibodies-positive pregnant women at 30 weeks of gestational age. This fact may also indicate maternal acquisition of re- or new chlamydial infection in later part of pregnancy. Intrauterine *C. trachomatis* infections acquired near the time of labor were considered to be associated with perinatal complications.

The serovars that we identified from Japanese infants and pregnant women were similar to those reported in other studies from non-trachoma-endemic areas and were thought to be mainly urogenital tract-origin[4,5]. Similar results were also obtained from the study of adult inclusion conjunctivitis in Japan[10]. Even some of serovars originally associated with endemic trachoma are occasionally detected in urogenital infection. Antigenic variations of *C. trachomatis* were found among the strains from nasopharyngeal, conjunctival and endocervical origins.

Erythromycin and clarithromycin were thought to be not toxic for fetuses and effective for the treatment of endocervical infection of *C. trachomatis*. Some serological variants of *C. trachomatis* may have different pathogenicity or drug-sensitivity from classic serotypes. Early diagnosis and appropriate treatment of chlamydial infections may reduce perinatal complications. Antigen detection of *C. trachomatis* from the endocervix has been utilized widely for the purpose of the screening of chlamydial infections during pregnancy. These tests are easily performed and less costly but have lower sensitivities than culture and have low positive predictive values in low prevalence populations such as Japan[11].

Time of onset of respiratory tract symptoms of the five infants in the present study was within 2 weeks after birth. One infant was born by Caesarean section. Diagnosis of

neonatal chlamydial infections was obtained by antigen or DNA detection from nasopharyngeal swabs and by detection of serum IgG and IgM antibodies to *C. trachomatis*[2,3]. However, chlamydial antigen was not detected from any endocervical specimens of mothers.

Control programs emphasizing early diagnosis, targeted screening, and effective treatment will have led to an eventual decline in the incidence of chlamydial infections[12,13]. Entirely new approaches to prevention and treatment of chlamydial infections in infants seem to be necessary, including antimicrobial interventions and the development of a vaccine strategy.

References

1) Numazaki K, Wainberg MA and McDonald J : *Chlamydia trachomatis* infections in infants. Can. Med. Assoc. J. 1989 ; 140 : 615–622.

2) Niida Y, Numazaki K, Ikehata M, Umetsu M, Motoya H and Chiba S : Two full-term infants with *Chlamydia trachomatis* pneumonia in the early neonatal period. Eur. J. Pediatr. 1998 ; 157 : 950–951.

3) Numazaki K and Niida Y : Two cases of intrauterine *Chlamydia trachomatis* infection. Antimicrobics and Infectious Diseases Newsletter 2000 ; 18 : 6 –8.

4) Ikehata M, Numazaki K and Chiba S : Analysis of *Chlamydia trachomatis* serovars in endocervical specimens derived from pregnant Japanese women. FEMS Immunol. Med. Microbiol. 2000 ; 27 : 35–41.

5) Numazaki K, Ikehata M, Chiba S, Suzuki K and Hashimoto N : Unclassified serovars of *Chlamydia trachomatis* isolated from Japanese infants. Clin. Microbiol. Infect. 1998 ; 4 : 519–523.

6) Numazaki K and Chiba S : Diagnostic value of rapid detection of *Chlamydia trachomatis* by using amplified enzyme immunoassay in infants with respiratory infections. Diagn. Microbiol. Infect. Dis. 1993 ; 17 : 233–234.

7) Numazaki K, Chiba S, Niida Y, Komatsu M and Hashimoto N : Evaluation of diagnostic assays for neonatal and infantile chlamydial infections. Tohoku J. Exp. Med. 1993 ; 170 : 123–129.

8) Numazaki K, Chiba S, Kogawa K, Umetsu M, Motoya H and Nakao T : Chronic respiratory disease in premature infants caused by *Chlamydia trachomatis*. J. Clin. Pathol. 1986 ; 39 : 84–89.

9) Numazaki K, Ikehata M, Akashi E, Kusaka T and Chiba S : Seropositivity to *Chlamydia trachomatis* during pregnancy and perinatal complications. J. Infect. Chemother. 1998 ; 4 : 28–31.

10) Numazaki K : Oculogenital transmission of *Chlamydia trachomatis*. Int. Med. J. 2000 ; 7 : 61–62.

11) Numazaki K, Niida Y and Chiba S : Antigen detection of *Chlamydia trachomatis* from the

endocervix is not enough for screening of perinatal complications. Am. J. Obstet. Gynecol. 1997 ; 174 : 951–952.

12) Numazaki K, Ikehata M, Chiba S and Aoki K : Reduction of trachoma in absence of a disease–control programme. Lancet 1997 ; 350 : 447–448.

13) Numazaki K : Current problems of *Chlamydia trachomatis* infections in Japan. Int. Med. J. 1999 ; 6 : 69–74.

3．市民(親や子)教育/啓蒙・コミュニケーション
―麻疹根絶に向けての取り組みを中心に―

国立感染症研究所　ウイルス第三部　麻疹室室長
沼　崎　　啓

小児科臨床別刷
58：2005―12月号

3．市民（親や子）教育/啓蒙・コミュニケーション
―麻疹根絶に向けての取り組みを中心に―

国立感染症研究所　ウイルス第三部　麻疹室室長　沼崎（ぬまざき）　啓（けい）

KEY WORDS ▶ 麻疹根絶，予防接種，市民教育/啓蒙

はじめに

麻疹は現在でも，世界中で発展途上国を中心に毎年3,000万人以上の罹患患者と87万5千人の死亡例（致死率3～5％）が発生しているものと推計されている[1)2)]。WHOは国連児童基金，米国CDCとともに2000年に「麻疹による死亡率の減少と地域的な排除のための世界麻疹排除対策戦略計画」を策定した。すでに南北アメリカ大陸，ヨーロッパ，南アフリカや中近東の一部の国々では，徹底的なワクチン接種と監視活動（サーベイランス）を実施してほぼ根絶に近い状態が達成されつつある。

日本を含むWHO西太平洋地域（WPRO）は最も対策の遅れた地域とされ，麻疹根絶の目標達成時期さえも設定されていない状況にあったが，現在2012年を一応の達成目標とする案が検討されている。わが国でも平成18年4月1日から予防接種法に基づく麻しんおよび風しんに係わる定期の予防接種において乾燥弱毒生麻しん風しん混合ワクチンの2回接種が実施されることになった。

本稿では世界的な麻疹根絶に向けての取り組みを中心に，感染制御の観点から市民（親や子）教育/啓蒙・コミュニケーションについて記載する。

Ⅰ．疾病としての麻疹の概要

1．麻疹ウイルスと病原性

麻疹ウイルスはparamyxovirus科morbillivirus属に属し，直径100～250nmのエンベロープを有する一本鎖RNAウイルスである。AからHのタイプに分類され，現在genotypeは22種類報告されており，ワクチン株はAである。麻疹ウイルスのレセプターは1993年に補体調節蛋白であるCD46（membrane cofactor protein）と発表された[3)]。CD46はヒトのすべての有核細胞に発現しており，サルではよく似たホモローグが赤血球にも認められるため，麻疹ウイルスのサル赤血球凝集反応が起こると説明されていた。2000年，Tatsuoら[4)]により新たなレセプターとしてリンパ組織系に特異的に発現するSLAM（signaling lymphocyte activation molecule；CD150）の存在が報告された。SLAMは未熟胸腺細胞，活性化されたリンパ球・単球，成熟樹状細胞に発現し，リンパ球の活性化とIFN-γ産生制御を誘導すると報告されている。

〒208-0011　東京都武蔵村山市学園4-7-1

エンベロープ蛋白のうち，主にF（fusion）蛋白とH（hemagglutinin）蛋白が麻疹の病原性に関与するが，F蛋白はウイルスと宿主細胞の膜融合を引き起こし，宿主細胞へのウイルスの侵入を可能にすることが知られている。1980年代の流行から明らかになったH遺伝子の変異は，1990年代になってF遺伝子に及んでいる。

最近の流行株は1950年代の株との間にH遺伝子で50～60塩基（アミノ酸では16～18カ所），F遺伝子では30～33塩基（アミノ酸で2～3カ所）に置換が起こっている。わが国で分離された麻疹ウイルスの遺伝子型は，2001年にはD5型（沖縄ではD3型）が大部分で，中国や韓国の分離株の主流であるH1型は川崎と東京で分離されていたにすぎなかった。しかし，2002～2003年は全国各地でH1型が分離された。

麻疹ウイルスは後述のカタル期の患者よりの飛沫，鼻汁などを介して気道，鼻腔および眼の粘膜上皮に感染する。麻疹ウイルスは感染後2～4日間，気道粘膜上皮の局所で増殖した後にリンパ球，マクロファージなどに感染して所属リンパ節に運ばれ，そこで増殖する。ウイルスはその後，白血球に感染したままで血流中に入り第一次ウイルス血症をきたす。ウイルス感染は侵入門戸付近から全身のリンパ節に拡大し，第二次ウイルス血症を生じ，臨床症状が出現する。感染力が強く，初感染時の不顕性感染は通常なく，一過性の免疫抑制状態を誘導する。ツベルクリン反応減弱などの細胞性免疫能の低下と，それに伴う細菌の二次感染による合併症，結核の顕性化は古くよりよく知られている。ウイルスは熱，紫外線，酸，アルカリ，エーテル，クロロホルムによって速やかに不活化される。空気中や物体表面では存在時間は短い。

2．麻疹の臨床像

i）前駆期（カタル期）

麻疹は一般に感染後10～12日の潜伏期を経て発症する。38°C前後の発熱が2～4日間続き，倦怠感や不機嫌をが続き，咳嗽，鼻漏，くしゃみなどの上気道症状と結膜充血，眼脂，羞明などの結膜炎症状が出現する。乳幼児では下痢，腹痛などの消化器症状を伴うことも多い。特徴的な発疹出現の1～2日前に頬粘膜の臼歯対面にコプリック斑と呼ばれるやや隆起し紅暈に囲まれた約1㎜径の白色小斑点が出現する。コプリック斑の確認は診断的価値が高いが，発疹出現後2日目の終わりまでに消失する。口腔粘膜は発赤し，口蓋部には粘膜疹がみられ，しばしば溢血斑を伴う。

ii）発疹期

カタル期での発熱が1°C程度下降した後，半日くらいのうちに再び二峰性発熱と呼ばれる39.5°C以上の高熱が出現する。同時に特徴的な発疹が耳後部，頸部，前額部より出現し，翌日には顔面，体幹部，上腕におよび，2日後には四肢末端にまで拡大する。発疹が全身に広がるまでは39.5°C以上の高熱が3～4日間持続する。発疹は当初，鮮紅色扁平であるが，まもなく皮膚面より隆起し，融合して不整形斑状の斑丘疹となる。また発疹は指圧によって退色し，一部には健常な皮膚を残す。その後発疹は暗赤色となり，出現の順序で退色する。発疹期には上気道，結膜炎症状は一層強くなり，特有のいわゆる麻疹様顔貌を呈する。

iii）回復期

発疹出現後3～4日間続いた発熱も回復期に入ると解熱し，全身状態が改善する。発疹は退色し，色素沈着がしばらくの間残り，僅かの糠様落屑を認める。合併症のないかぎり，発症後7～10日には回復する。患者の気道からのウイルス分離は，前駆期（カタル期）の発熱時から可能となるが，発疹の色素沈着以後は分離されない。感染力はカタル期が最も強い。

iv）合併症