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

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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].

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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

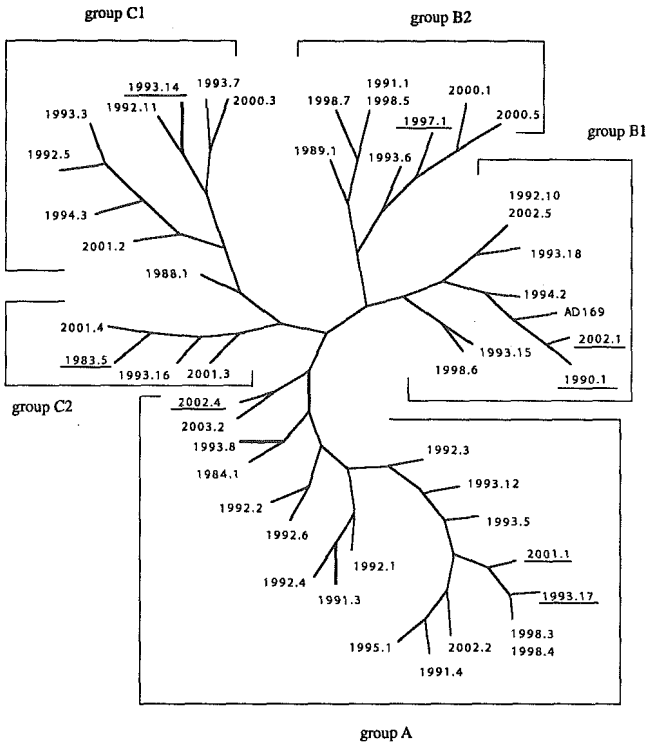

Fig. 1. Unrooted phylogenetic dendrogram showing the relationship of 48 human cytomegalovirus (HCMV) strains with respect to α sequence polymorphisms. AD169 strain is included. Numbers with underlines indicate isolates from congenital infection.

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 α 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%

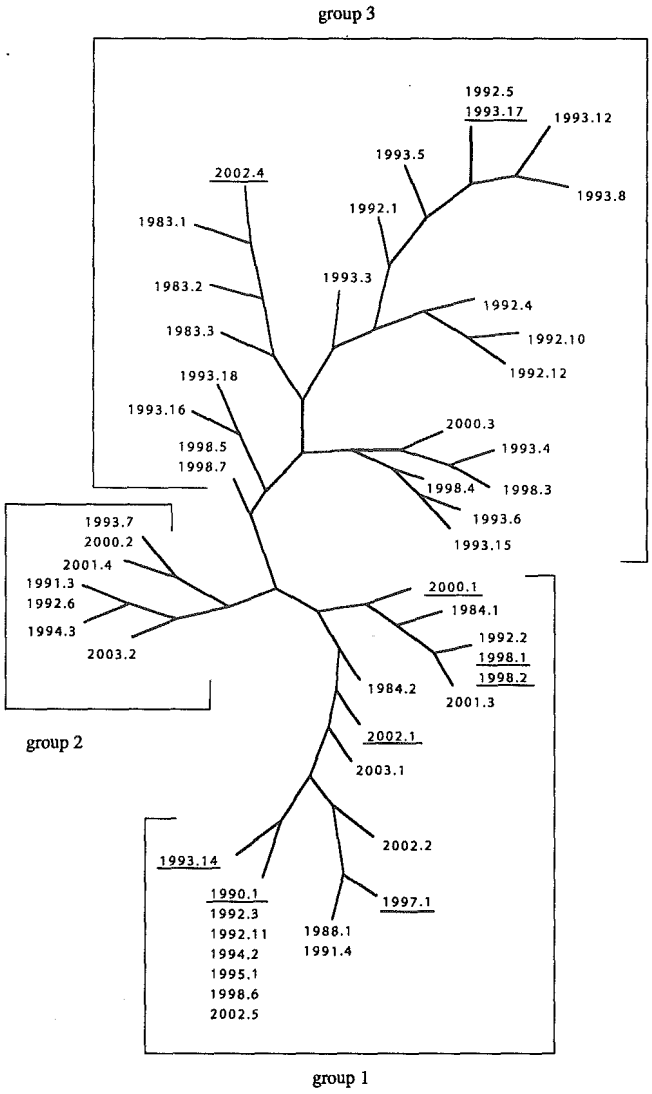

Fig. 2. Unrooted phylogenetic dendrogram showing the relationship of 52 HCMV strains with respect to *UL144* gene variability. Numbers with underlines indicate isolates from congenital infection. Group designations conform to the scheme proposed by Lurain et al. [1999].

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.

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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―

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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。

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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

麻疹の二大死因は肺炎と脳炎であり，5歳以下あるいは20歳以上で多い。合併症は年少ほど死に至る危険性が高い。ウイルス性肺炎は病初期に認められるが，発疹期を過ぎても解熱しない場合には細菌性肺炎を考慮すべきとされる。起因菌としては，肺炎球菌，インフルエンザ菌，化膿レンサ球菌，黄色ブドウ球菌などが多い。巨細胞性肺炎は成人の一部，あるいは細胞性免疫不全状態時にみられる特徴的な肺炎である。一般に予後不良であり，死亡例も多い。

中耳炎は麻疹患者の約5～15%にみられる最も多い合併症の一つである。細菌の二次感染により生じる。乳様突起炎を合併することがある。喉頭炎および喉頭気管支炎の合併症も多い。心筋炎，心外膜炎を合併することもある。麻疹の経過中半数以上に，一過性の非特異的な心電図異常がみられる。

1,000例に0.5～1例に脳炎などの中枢神経系合併症を発症する。発疹出現後2～6日に発症することが多いが，麻疹の重症度と脳炎発症には相関はない。患者の約60%は完全に回復するが，20～40%に精神発達遅滞，けいれん，行動異常，神経聾，片麻痺，対麻痺などの後遺症を残し，致死率は約15%である。

亜急性硬化性全脳炎（subacute sclerosing panencephalitis, SSPE）は麻疹ウイルスに感染後，学童期などに発症する特徴的な中枢神経疾患である。知能障害，運動障害が徐々に進行し，ミオクローヌスなどの錐体・錐体外路症状を示す。発病までの期間は，麻疹罹患例で平均7年を要し，麻疹ワクチン接種例では平均3年で発病する。麻疹ウイルスの中枢神経系細胞における持続感染により生じるが，本態は不明である。

麻疹初感染時の症状はほとんどが軽症であるが，その後もウイルスのM（matrixa）蛋白，H蛋白，F蛋白の発現に欠損が認められる欠損ウイルス粒子として存在し続ける。in situ reverse transcriptase-PCR（in situ RT-PCR）により，ウイルスRNAが患者の神経系細胞や血管内皮細胞から検出されたという報告もある。発症から平均6～9カ月で死の転帰をとることもある。発生頻度は，麻疹罹患者10万人に1人，麻疹ワクチン接種者100万人に1人である。診断は，麻疹の既往歴の確認，血清中の麻疹抗体価（HI, CF抗体価）の異常高値（＞1：1280），髄液中の麻疹抗体の検出などによる。

3．特殊な麻疹の病型

i）修飾麻疹（modified measles）

不十分な免疫下に麻疹ウイルスが感染した場合は軽症の不全型麻疹を発症することがある。潜伏期が14～20日に延長し，前駆期症状は軽微か欠落し，コプリック斑は出現しないことが多い。発疹は急速に出現するが，融合しない。通常合併症はなく，経過も短い。要因としては，移行抗体の残存，γ-グロブリン投与，secondary vaccine failure（SVF）状態などがある。

ii）異型麻疹（atypical measles）

現行の麻疹ワクチン接種以前は，生ワクチンの発熱率が高く，不活化ワクチンと併用されていた時期があった。不活化ワクチン接種2～4年後に自然麻疹に罹患した際に異型麻疹がみられることがある。4～7日続く39～40°C台の発熱，肺炎，肺浸潤と胸水貯溜，発熱2～3日後に出現する特徴的な非定形発疹が主な症状で，コプリック斑を認めることは少ない。全身症状は1週間くらいのうちに好転し，発疹は1～3週で消退する。回復期の麻疹HI抗体価は通常の麻疹に比して著明な高値をとる。

4．麻疹の診断と治療

ウイルス分離，麻疹特異的IgM抗体の検出，急性期と回復期のペア血清での麻疹IgG抗体の有意な上昇の確認によって実験室内診断は可能である。わが国では臨床症状のみで診断することが多かったが，抗体測定には，赤血球凝集抑制法（hemagglutination inhi-

bition：HI)，中和法，ゼラチン粒子凝集法（particle agglutination：PA)，ELISA 法などが用いられている[5]。ウイルスは通常，咽頭拭い液，血液などから分離され，カタル期から発疹出現後3日以内の分離率が高い。

ウイルス分離には従来ヒト腎細胞や Vero 細胞を用いて行われてきたが，細胞変性効果（CPE）が出現するまでに数週間を必要とした。マーモセットのB細胞を EB ウイルスでトランスフォームした B95a 細胞[6]や SLAM 遺伝子を組み込み，発現させた Vero/SLAM 細胞[4]ではウイルス野生株が高率に早期より分離される。麻疹では特異的な治療法は確立されていない。

II．麻疹の予防とワクチン

麻疹の感染力は極めて強く，度々集団発生を引き起こしてきた。麻疹は学校保健法に基づく第二種の伝染病に属し，登校基準としては，「発疹に伴う発熱が解熱した後3日を経過するまで出席停止とする」とされている。

国内の麻疹ワクチンは，1966年から，不活化ワクチン（K：killed vaccine）と生ワクチン（L：live vaccine）の併用法（KL 法）によって接種が開始された。これはLワクチン接種前にKワクチンを接種することにより発熱の軽減化などが考えられたためである。Kワクチンによって感作された後に自然麻疹に罹患したときに，異型麻疹の発生が問題となった。また，Kワクチンを先に接種することによりLワクチンによる抗体獲得が得られない場合があることなどから，KL の併用は中止となった。

1969年以降は高度弱毒生ワクチン（FL：further attenuated live vaccine）の単独接種に切り替えられた。1978年から開始された定期麻疹ワクチン接種は FL ワクチンが採用された。現在わが国で市販されているワクチンは，武田薬品工業の Schwarz-FF8 株，北里研究所の AIK-C 株，阪大微研の CAM 株，千葉血清研の TD97 株の4社由来株ワクチンである。Enders の分離した Edmonston 株由来の AIK-C 株，Schwarz-FF8 株と，阪大微研で分離した田辺株由来の CAM 株，TD97 株を起源としており，最終製品はニワトリ胎児胚細胞（CE 細胞）で増殖したウイルスを含む培養上清を精製して作られている。これらのワクチンは凍結乾燥品であり，使用時添付の溶解液（蒸留水）0.7mℓで溶解後，0.5mℓ（力価5,000TCID50/0.5mℓ以上）を皮下接種する。

その後，安定剤として含まれていたゼラチンがアナフィラキシーショックを含む重篤なアレルギー反応の原因となることが判明し，1996年から1998年にかけて除去あるいは低アレルゲン性ゼラチンへの変更等の改良が加えられた。

1989年，わが国においても MMR ワクチン（統一株）が導入され，定期接種のワクチンとして麻しんワクチン，MMR ワクチンのどちらを接種してもよいことになった。ところが，MMR ワクチン中に含まれるおたふくかぜワクチン株による無菌性髄膜炎の多発が問題となり，製造メーカー独自の株を使用した自社株 MMR ワクチンへの切り替えが行われた。これも無菌性髄膜炎多発の解決には繋がらず，MMR ワクチンは1993年に接種中止となった。

現行麻しんワクチンによる免疫獲得率は95％以上と報告されている。接種後の反応としては発熱が約20～30％，発疹は約10％に認められる。いずれも軽症であり，ほとんどは自然に消失する。

1歳前にワクチン接種を受けた場合は，1歳以降に再接種（この場合は定期接種として実施）する必要がある。また，γグロブリンを投与された後は，6カ月未満の乳児と同様の理由で効果が得られないため，3カ月間は接種を行わない。川崎病などの治療で大量療法を受けた場合には，6カ月間あける必要が

表1　感染症法に基づく麻疹に関する報告のための基準

○診断した医師の判断により，症状や所見から当該疾患が疑われ，かつ，以下の3つの基準をすべて満たすもの．
1．全身の発疹(回復期には色素沈着を伴う)
2．38.5℃以上の発熱
3．咳嗽，鼻汁，結膜充血などのカタル症状
なお，コプリック斑の出現は診断のための有力な所見となる

○上記の基準は必ずしも満たさないが，診断した医師の判断により，症状や所見から当該疾患が疑われ，かつ，病原体診断や血清学的診断によって当該疾患と診断されたもの．

ある。

2003年の「感染症の予防および感染症の患者に対する医療に関する法律」(感染症法)改正に伴い，麻疹は4類感染症から5類感染症に分類変更になった。全国約3,000カ所の小児科定点より毎週報告がなされているが，報告のための基準は表1の通りである。

III．WHOの麻疹根絶計画と対策の現状

WHOは，毎年世界で3,000万人以上の麻疹患者と875,000人の麻疹による死亡者が発生しているものと推計している。この死亡数は，全世界の感染症による死亡数14,025,000人のうち，6.24%を占め，単独の病原体としては最大の死亡原因である。

2000年，全世界において麻疹による死亡率を低下させるために，WHOは国連児童基金(UNICEF)，米国疾病管理予防センター(CDC)とともに，「麻疹による死亡率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(Global Measles Strategic Plan for Measles Mortality Reduction and Regional Elimination)」を策定した[1]。具体的な目標を設定し，死亡率減少と地域的排除のための活動を進めるための枠組みを示し，1回目の麻しんワクチン接種に加えて，補足的な予防接種活動として，すべての小児に2回目の接種機会を与えることを勧奨している。これにより，これまで接種を受けなかったかあるいは1回目の接種で免疫を獲得しなかった児のすべてに対して麻疹に対する免疫をつけることが可能である。また，この対策活動を行うにあたり，風疹の予防接種およびサーベイランス活動を組み入れていくことが勧奨されている。

現在わが国は，中国，インド，その他の途上国とともに，第一段階である制圧(control)期に含まれている。オーストラリアなどのオセアニア諸国の多くは第二段階の集団発生予防(outbreak prevention)期に，またアメリカ大陸，ヨーロッパ，南アフリカや中近東の一部は，すでに排除(elimination)期としての対策が進んでいる。

米国，カナダなど内因性の麻疹伝播を排除している国では，ワクチン接種率が95%を超えている(米国のデータは2回接種の接種率が91%，1回接種の接種率は96%)。この高い接種率は入学・入園時での麻しんワクチン接種がその条件として要求されていることが大きいと考えられる。これらの国での患者発生はほとんどが輸入例であり，米国の輸入例中第一位は日本からの輸入例である[7][8]。

イギリス，フランス，イタリア，ドイツなどでも，ワクチン接種の方針はMMRワクチンの2回接種であるが，その接種率は80～90%で，年間の麻疹患者は数千から一万人程度の発生があり，毎年10人までの死亡が報告されている(表2)。

途上国においては，まず可能な限り定期接種において，1回のワクチン接種を徹底することを目標としている。しかし，2回接種方針をとっても定期接種のみでは，全体の接種率を上げることは不可能である。そこで補足的予防接種キャンペーンを行い，対象期間中，対象地域におけるすべての小児に対して

表2　先進国での麻疹患者発生数と予防接種率

国　名	接種方法	接　種　率	患　者　数	死亡数
米　　国	MMR2回接種	91%(19～35カ月児，2回接種)	100人	2001年2人
		以前の1回接種の接種率は96%	人口10万当たり 0.04	
カ ナ ダ	MMR2回接種	96%(2歳児における接種率)	28人(確定診断例，1999年)	データなし
イギリス	MMR2回接種	88%(1999/2000年に2歳になる児のコホート)	72人(確定診断例，2001年)	2人
フランス	MMR2回接種	84.2%(2歳児 2000年)	10,000人(推計値 2000年)	10人以下
		90%(6歳児 2001年)		
ド イ ツ	MMR2回接種	84.6%	人口10万当たり 46.8	データなし
イタリア	MMR2回接種	80%程度(2歳児，2000年)	人口10万当たり 60	7人

麻しんワクチンを一斉接種することにより，予防接種率を上げようとしている。

2回接種の目的は，1回接種で免疫獲得が困難なもの（primary vaccine failure，PVF）に獲得させることと1回目で獲得された免疫を増強させる（学校等での集団発生を予防する）という2点である。その結果，1回接種で免疫を獲得したが，年数の経過と共に免疫の低下が起こり修飾麻疹，非典型麻疹として発症するSVFに対してもある程度の効果が期待できる。

麻疹の潜伏期間中に出国した日本人海外旅行者が現地で発症した事例が報告され，日本は麻疹の輸出国であるとの不名誉な指摘も受けている。今後の問題点としては，SVFの増加，妊婦麻疹およびそれに関連する新生児麻疹の発生，流行地域への旅行時の罹患・再罹患などが考えられる。

これらの問題の解決のためには95%以上のワクチン接種率の向上後に適切な時期に麻しんワクチンを追加接種する必要がある。また麻しんワクチンの改良，次世代ワクチン開発のための研究を進めることも重要である。世界的には麻疹制圧（control）から集団発生予防（outbreak prevention），排除（elimination）にむけ目標が設定され，さらには根絶（eradication）に関する議論がなされている（表3）。

表3　WHOが区分している麻疹排除に向かう各段階

○第一段階：制圧(control)期
麻疹は恒常的に発生しており，頻回～時に流行が起こる状態，麻疹患者の発生，死亡の減少を目指す時期

○第二段階：集団発生予防(outbreak prevention)期
全体の発生を低く抑えつつ集団発生を防ぐことを目指す時期

○最終段階：排除(elimination)期
国内伝播はほぼなくなり，根絶(eradication)に近い状態

1歳未満の麻疹は死亡を含む重症化率が高いため，定期接種として，生後9カ月前後を麻しんワクチン接種対象年齢にしている国々が途上国を中心に少なくない。麻疹対策の進んでいない地域では，多くの乳児が麻疹患者と接触する可能性が高く，乳児への早期接種は乳児感受性者群の割合を減少させる効果が期待される。

麻疹罹患の危険性が少ない先進国では，1歳以上を接種対象としている。WHOでは，生後9カ月以下の児に罹患する可能性が高い流行状態であればあくまで一時的に，生後6カ月児からの乳児への接種も可能としてい

る。CDC では，麻疹に罹患する危険性が高ければ，生後6カ月より麻しんワクチン接種を行い得るとしているが，1歳未満で接種を受けた場合には生後12～15カ月で再接種を行うべきであるとしている。

Ⅳ．わが国の麻疹の現状と対策

わが国の年間患者数はこの10年間で明らかに減少しているものの，いまだに定点届け出数25,000人前後，推計で10～20万人程度の発生がある[2]。年齢別報告数は，2歳以下が全報告数の半数を占めている。わが国の小児へのワクチン接種率は最近全国平均で80％に達したが，地域によっては50～60％と低い状況にある。麻疹に感染することなく，麻しんワクチン未接種のまま成長した成人も麻疹（成人麻疹）の増加も問題となっている。

わが国の麻疹患者は，2001年には年間28.6万人と推計され，米国の116人（2001年）と比較すると約2,500倍の発生率であった。2001年に報告された麻疹患者の年齢は，1歳23％，0歳15％，2歳10％で，0～2歳が報告患者の47％を占めたのに対し，2003年は，0歳は16％で変わらなかったものの1歳19％，2歳7.3％に減少した。3～9歳は不変であった。

年長児の麻疹の割合は2001年10～14歳11％，15～19歳3.5％，20歳以上2.1％であったのに対し，2003年はそれぞれ15％，6.3％，3.7％に増加した。1984年や1991年の流行後は，患者数が少なくなると1～4歳の割合が増加し5歳以上の割合が減少しているが，2001年の流行後は，2002年，2003年と患者数が減少したにもかかわらず，1，2歳の割合が減少して，5歳以上の割合が増加している。ゼラチン粒子凝集反応法（PA 法，1：16以上陽性）による2002年度の1歳の麻疹抗体保有率は73.2％で，前年度（43.9％）に比べて上昇していたが，2003年度は61.9％と低下している。

1歳児の麻しんワクチン接種率も45％から78％に増加しており，2001年から始まった「1歳になったらすぐに麻しんワクチン接種を」のキャンペーンが功を奏したと考える。一方，0歳児の抗体陽性者は0～5カ月児で83％から67％，6～11カ月児で32％から14％に減少し，移行抗体の消失時期が早くなっていることが推定された。麻疹はこの10年間，春季を中心とする流行を繰り返してきたが，2004年は全国単位では，流行と呼べる程の発症者の増加はみられなかった。

現在のわが国では乳児が罹患するリスクは途上国と同程度に高いと思われるが，死亡率，重症化のリスクは先進国と同程度に低いと考えられる。わが国においても，麻疹流行時の生後6～11カ月児への予防接種は個人予防，集団予防の視点から緊急接種としての必要性が検討されるべきであるが，この年齢における現行ワクチンの効果および安全性は十分評価されてはいないのが現状である。平常時における乳児への接種の導入については更に継続的な検討が必要である。

平成18年4月1日から予防接種法に基づく麻疹および風疹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。
麻しんおよび風しんワクチンの接種においては，予防接種法施行令で定める対象者は，第1期月齢12カ月～24カ月に達するまで，第2期5歳以上7歳未満で2期は就学1年前から就学前日までの間にあるものとされた。

経過措置としては平成18年4月1日前に麻しんまたは風しんのいずれか一方の単抗原ワクチンの定期接種を受けたものに関しては第2期の接種対象としないものとされた。平成18年4月1日以降に5歳以上7歳未満となるものでいずれの予防接種を受けていないものは第2期の予防接種の対象者となった。

特定の地域において，複数の麻疹患者が短期間に確認された場合には，急速な感染拡大

表4　麻疹根絶のためのWHO認証麻疹研究・検査施設の役割

1）ウイルスの伝播の監視と証明
流行の確認，流行初期の臨床診断の確認，症例の確認，実験室内診断に伴う確定診断による認定，麻疹ウイルス株の同定と分離株の遺伝子的特徴の確認
2）集団における感受性者の監視
予防接種推進に伴う麻疹感受性者の年齢分布の確定，広報活動と反響に関する評価
3）ワクチン接種後の有害事象に関する研究
4）標準的実験室的手法による各国麻疹検査室の評価
5）各国麻疹検査室の技術維持のための講習会・ワークショップ等の開催
6）実験室間ネットワークおよび研究者間の情報交換体制の構築

が懸念され，流行対策の措置が必要である。家庭や集団生活の場（保育園，幼稚園，学校，職場など）において麻疹に関する知識の普及をはかるとともに，患者と感受性者との接触を減らすように務めるなど，小児を取り扱う医療機関において麻疹の再認識を深める必要性も指摘されている。

同一集団から麻疹患者が発生した場合には，個人予防の視点から，成人を含む感受性者に対して麻しんワクチン接種，ガンマグロブリン製剤の緊急避難的投与等の迅速な感染防御対策も必要である。

V．麻疹根絶における検査・研究施設の役割

麻しんワクチン接種後に野外ウイルスに暴露されると，症状は出現しないものの，感染することにより免疫力を保持あるいは高めるブースター効果があると考えられる。一時期のわが国のように，患者数がある程度減少した状況では，その機会が少なく，年長者の麻疹患者が増加する現象が認められる。これらの対策として，前述のようにわが国でもMRワクチンの2回定期接種が決定されたところである。

麻疹対策においてわが国は，自国の麻疹対策を見直し，WHOの麻疹根絶対策戦略計画による徹底的なワクチン接種と監視活動（サーベイランス）強化を推進し麻疹の排除，根絶を図る必要がある。このように麻疹ウイルスを効果的に封じ込め，根絶へと導くためには，ワクチンの二回接種とともに，疫学的監視体制とウイルス学的および遺伝子学的特性に基づいた科学的監視体制の充実が不可欠である[9]。

また西太平洋地域の各国に対し技術支援を行い，2012年根絶の目標達成にむけて国際的責務を果たす必要がある。元来，わが国の麻疹に関する基礎的研究レベルは世界のトップクラスとの評価を受けていたので，科学技術および研究成果の面でも西太平洋地域のみならず世界的貢献が期待されている。

国立感染症研究所麻疹室は，これまでの麻疹研究の実績から麻疹根絶の目標達成のための検査研究施設として，WHOの国家麻疹検査施設（National Measles Laboratory），地域麻疹レファレンス検査施設（Regional Reference Laboratory），世界麻疹特別検査施設（Global Specialized Laboratory）の三つに指定されることになった。サーベイランスの中心研究施設であるWHO認証麻疹研究・検査施設としては表4の各事業を積極的に推進する必要がある。

国内の麻疹対策のためには中央および地方の公衆衛生担当者が，十分にサーベイランスを活用することも重要である。麻疹の流行的発生にあたっては，適正な疫学調査を行い，原因の検討，対策の立案，実施を行う必要がある。

おわりに

国内の麻疹対策はもちろんのことである

が，わが国は西太平洋地域の各国に対し技術支援を行い，2012年根絶の目標達成にむけて国際的責務を果たす必要がある。また科学技術および研究成果の面でも西太平洋地域のみならず世界的貢献も期待されている。

以上のことから，多数の麻疹野生株を収集し，そのゲノムの解析を進め，変異による病原体の構造や機能の変化，病原性の変化を解明するとともに，信頼度の高い迅速診断法ならびに有効性と安全性の高い新たなワクチンを開発する必要がある。麻疹根絶にむけての取り組みにおいて感染制御の観点から市民の教育，啓蒙活動が最も重要であることは言うまでもない。

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