Early acquisition of cytomegalovirus infection

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SUMMARY Two hundred and fifty three infants were screened for cytomegalovirus (CMV) in the urine at birth and were followed up at regular intervals for one year. Twelve per cent (of 249) were excreting virus at 3 months, and 20% (of 234) at 12 months. In all cases infection was subclinical. The major factors determining risk of acquiring infection were the mother's serological state and whether the infant was breast fed. There was no association with social class, mother's age, or whether the child had been in a special care baby unit or a postnatal ward. By one year 33% (of 123) of infants of seropositive mothers had acquired CMV infection compared with 4% (of 123) born to seronegative mothers. Twenty per cent (17) of seropositive women who breast fed had virus isolated from their breast milk on at least one occasion, and 76% (13) of their infants became infected. In four mother-infant pairs comparison of CMV isolates from the mother's milk and the child's urine was made by restriction endonuclease digestion; in each pair infection had apparently occurred with the same strain of virus. All 13 infected infants followed up for three years were still shedding virus.

Infection with CMV is common in infancy, and virus shedding persists for years. Congenital infection cannot be distinguished from acquired infection unless the presence of CMV in the urine is identified within three or four weeks after birth, even when clinical problems suggestive of congenital infection are present.

Congenital cytomegalovirus (CMV) infection is the most common intrauterine infection and an established cause of handicap.¹⁻⁴ The prevalence of congenital infection in the United Kingdom is 3–4/1000 live births.⁵ Follow up studies have shown that 10% of congenitally infected infants will have permanent neurological sequelae.⁴ In contrast to congenital infection, the prevalence and importance of CMV infection acquired after birth has not been well documented in the United Kingdom. In September 1982 a prospective study was set up to investigate the prevalence of CMV infection acquired in the first year of life in a London population and to investigate its role, if any, in the cause of childhood illness. We describe here the acquisition of CMV infection in the first year of life and relate this to maternal CMV antibody state and to possible sources of infection, such as breast milk.

Patients and methods

Study population. During one year from 1 September 1982, 269 infants from the Charing Cross Hospital maternity unit were enrolled in the study.

They comprised all infants admitted to the special care baby unit for more than 24 hours (102 infants) and 167 randomly selected from the postnatal wards. All children were screened at birth for congenital infection, and during this period two infants had CMV in their urine and were therefore congenitally infected. They were not enrolled. Of the 269 infants enrolled, three from the postnatal wards and 13 from the special care baby unit were lost to subsequent follow up: 11 had left the district or were transferred to other hospitals, four died while in the special care baby unit, and one mother refused to participate in the study. Thus 253 infants were available for follow up.

Infant follow up. A throat swab and a urine sample were taken from each infant in the first week of life and cultured for CMV. Samples were inoculated in duplicate into human embryonic lung cells, and CMV was identified by the appearance of typical cytopathic effects. Weekly urine samples were collected from all infants while they remained in hospital.

Details of all blood transfusions to the infants

were recorded and aliquots of blood from each transfusion tested for antibodies to CMV by complement fixation test.

After discharge from hospital arrangements were made for the research nurse (CJ) to visit the infants at home or see them in the paediatric clinic at six weeks, three months, eight months, and one year. In fact, the visits were on average at eight, 15, 37, and 55 weeks. At each visit a urine sample was taken and cultured for CMV. At the one year visit all children were examined by a paediatrician (KP), who carried out a Griffiths developmental assessment. At this visit a fingerprick blood sample was taken on to absorbent blotting paper and tested for antibodies to CMV by radioimmunoassay.

Of the 253 infants followed up to six weeks, 249 were seen at three months, 247 at eight months, and 234 at one year. In a separate study to check for how long CMV was present in the urine the 46 infected infants were followed up for longer periods, 13 for up to 36 months.

Information about mothers. As part of a continuing prospective study of CMV infection in pregnancy a short questionnaire was completed by interview for all women attending the antenatal clinic for the first time.⁵ Information on age, marital state, parity, race, country of origin, previous or current occupation, and husband's or partner's occupation was obtained. In addition a blood sample was taken and tested for the presence of antibodies to CMV by complement fixation test. A dilution of 1/10 was taken to indicate previous infection. Seronegative women were retested during pregnancy and at term to identify those in whom seroconversion occurred.

Breast milk samples were collected weekly from mothers while their infants remained in hospital and subsequently at each follow up visit if breast feeding was continued. These samples were inoculated into human embryo lung cells after they had been spun down at 4000 rpm for 20 minutes and the creamy layer had been removed.

Viral DNA of CMV isolates from the breast milk of four mothers and the urine of their infected infants was typed by restriction enzyme analysis.⁶

Statistical analysis. The effect of breast feeding and the mother's serological state on CMV acquisition was assessed by life table analysis. Four periods at risk were considered: 0-8, 9-15, 16-37, and 38-55 weeks. It was assumed that CMV has a three week latent period, as the earliest evidence of infection was found in urine samples taken when infants were 3 to 4 weeks old. Thus infants were considered to have been at risk for five weeks before the visit at the end of the eighth week. Separate life tables were

constructed by adding time at risk before the end of breast feeding to the breast feeding life table and subsequent time to the non-breast feeding life table. In this way four life tables were constructed, one for each combination of the mother's serological state and the feeding method. Mantel-Haenszel odds ratios and their 95% confidence intervals were computed, stratified by time period.⁷ The odds ratio associated with maternal seropositivity (for example) is the amount by which seropositivity multiplies the odds of acquisition of CMV relative to the infants of seronegative mothers. The constancy of the odds ratios across the four time periods was assessed by the methods described by Breslow and Day.⁸

The life table method used here was only approximate because of slight variations in the time between visits and the assumption of constant risks within each time period.

Results

Of the 246 mothers, 10 were black, four Asian, and six oriental, the rest being white. The mother's serological state for CMV was known for 246 of the 253 infants seen six weeks after birth. The remaining seven had booked elsewhere for their antenatal care, and information on their CMV state was not available. Of the 246 mothers whose CMV state was known, half were seropositive; this rate is similar to that among women attending the antenatal clinic at this hospital.

As a check on the reliability of using virus isolation to define infection blood samples taken at 1 year of age were analysed for antibody to CMV. Antibodies were present in 45 of the 46 children who had excreted virus in their urine, and no additional children with antibodies from whom CMV had not been isolated were identified. The one seronegative child from whom virus had been isolated was first excreting CMV at 1 year, and seroconversion may not yet have occurred.

Table 1 shows the number of children excreting CMV at each age studied. The earliest positive urine cultures were obtained between 3 and 4 weeks. By one year 46 infants (20% of those sampled at this age) were excreting virus. The chances of acquiring

Table 1 Acquisition of cytomegalovirus infection

	Age at screening			
	6 Weeks	3 Months	8 Months	1 Year
No screened	253	249	247	234
No (%) excreting CMV	9 (3.6)	30 (12.0)	37 (15.0)	46 (19.7)

CMV infection, however, were far from uniform and depended on maternal characteristics (Table 2). The risk of acquiring CMV was much higher for children of seropositive mothers than for children of seronegative mothers. Breast feeding was also strongly related to acquisition of CMV. The mother's age and social class, however, showed no relation to the acquisition of infection. There was no important difference in the proportions of infants from the special care baby unit and postnatal wards who had acquired infection.

Only five of the 123 infants born to seronegative mothers became infected, and two of these had received seropositive blood while in the special care baby unit. One, who had been born at 40 weeks and weighed 3400 g, had been given 70 ml of seropositive blood on day 16 and was found to be shedding virus when 3 months old; urine samples taken up to six weeks had been negative. The other infant had been born at 26 weeks' gestation and weighed 650 g; he had received 12 ml of seropositive blood at birth, 10 ml when 2 weeks old, and 10 ml when 3 weeks old. Virus was first isolated from the urine at 8 weeks. Weekly urine samples up to then had been negative for CMV. The source of infection for the three other children of seronegative mothers, one of whom was first found to be shedding CMV at 8 months and two at 1 year, was unknown. None of the children had received day care. Interestingly, the mothers of the two children who first shed CMV when 1 year old were seropositive when they booked for subsequent pregnancies the following year. Maternal serological state was not otherwise

monitored and could have changed over the one year period. Assuming a 3% seroconversion rate in the seronegative groups,^{9 10} we would expect to find three or four maternal seroconversions.

As the major factors identified to be associated with acquisition of CMV infection were breast feeding and mothers' serological state the subsequent analysis was confined to these two factors. Figure 1 shows the rate of acquisition rate of CMV, defined as the number of new cases/1000/week. The rate was highest in the period between nine and 15 weeks.

Life table analysis showed that by one year 39% of infants of seropositive mothers who breast fed had become infected, compared with 24% of those who were bottle fed. Among the infants of seronegative mothers the percentages were 11% and 2% respectively. Infants with seropositive mothers had a far higher rate of infection than those with seronegative mothers, whether they were being breast fed (odds ratio 9.3, 95% confidence interval 3.6 to 24.0) or bottle fed (odds ratio 18.7, 95% confidence interval 4.3 to 80.0). The effect of maternal seropositivity on the acquisition of infection in breast fed infants tended to diminish over time ($\chi^2=7.5$, $df=1$, $p<0.01$).

Breast feeding was also associated with infection, though less strongly than the mother's seropositivity. Among infants of seropositive mothers the odds ratio of acquisition of CMV in breast fed,

Table 2 Factors associated with acquisition of CMV infection in first year

	No at risk	No (%) of infants acquiring CMV
<i>Mother's serological state:</i>		
Seropositive	123	41 (33.3)
Seronegative	123	5 (4.1)
Total	246	46 (18.7)
<i>Characteristics of seropositive mothers</i>		
<i>Social class:</i>		
Non-manual	56	19 (33.9)
Manual	65	20 (30.8)
Other	2	2 (100.0)
<i>Hospital unit:</i>		
Postnatal ward	80	28 (35.0)
Special care baby unit	43	13 (30.2)
<i>Maternal age (years):</i>		
<20	5	2 (40.0)
20-29	56	17 (30.4)
30+	62	22 (35.5)
<i>Breast feeding:</i>		
Never	36	7 (19.4)
<12 weeks	23	7 (30.4)
12 + weeks	64	27 (42.2)

Fig. 1 Acquisition rate of cytomegalovirus infection, by mother's serological state and method of feeding.

compared with bottle fed, infants was 2.3 (95% confidence interval 1.1 to 4.9). There was a similar result in infants of seronegative mothers (odds ratio 4.7), but the confidence interval was wide (1.0 to 22.0).

Source of maternal infection. Isolation of CMV from breast milk was intermittent. CMV was isolated from colostrum or milk samples collected from 17 of 87 (20%) mothers who had breast fed. The infants of 13 of 17 (76%) mothers who shed virus in breast milk acquired infection. One mother who did not breast feed at all was shedding CMV in colostrum, and her infant acquired infection at 1 year of age. CMV infection was not acquired by breast fed infants of four mothers despite virus in breast milk or colostrum. This included the infant of one mother from whose breast milk the virus was repeatedly isolated.

CMV was isolated from the urine of three women (1.2%) in the first week after delivery. Two of these women were also shedding virus in their breast milk, and both their children acquired CMV infection.

Four pairs of CMV isolates, from mother's

breast milk and their infant's urine, were typed for molecular relatedness by restriction endonuclease digestion analysis.⁶ In three pairs of isolates the breast milk samples had similar DNA fragments to those isolated from their respective infants' urine, indicating that infection had occurred with the same strain of CMV. The fourth pair showed only minor differences in the patterns, which were quite distinct from the major differences seen between epidemiologically unrelated strains of CMV.

Duration of viral shedding in infants with acquired CMV infection. Further urine samples were obtained at 18, 24, 30, and 36 months from those children infected during the first year. Figure 2 shows that only one of 25 infants excreting at 12 months and followed up until 30 months was not shedding virus in the urine; this same infant was shedding virus at 36 months. All 13 infants from whom a urine sample was collected at 36 months were shedding CMV in their urine.

Discussion

The prevalence of CMV infection acquired by children during the first year varies throughout the world, and in populations of different ethnic and social backgrounds it ranges from 8% to 60%.¹¹ In our study one fifth of children had acquired CMV infection by 1 year of age and 12% were already infected by 3 months. Previous cross sectional studies in the United Kingdom of children in hospital showed lower rates of infection.^{12 13} In a study of unselected admissions to acute medical, surgical, and paediatric wards in one hospital in 1968 CMV was isolated from 2.5% of 118 neonates, 9% of 32 infants aged 2.5 months, and 10% of 104 children aged 6 months to 4 years.¹² In the collaborative study,¹³ which was also cross sectional and based on hospital admissions, the prevalence of virus excretion was 1.8% in 508 infants aged under 3 months, 3.2% in 220 aged between 3 and 5 months, and 5.8% in 207 aged 6 to 11 months.

The overall rate of seropositivity in mothers is the most important factor determining the extent of early acquisition of CMV. In countries where a high proportion of women of childbearing age have antibodies to CMV the acquisition of early infection is high. In Finland in a population where 82% of women were seropositive, 32% of 148 infants acquired CMV infection during their first year.¹⁴ Most of these infants were infected by 4 months, and few acquired infection after this age. None of the infants of the 23 seronegative mothers became infected, whereas 38% of those born to seropositive mothers acquired infection. These results are

Fig. 2 Duration of viral excretion in infants with acquired cytomegalovirus infection.

comparable with those in our study, in which 34% of infants of seropositive mothers were infected by 1 year, most by 3 months. In Japan high rates of excretion of CMV were also reported¹⁵ in a population where the rate of seropositivity among women of childbearing age is over 90%.¹⁶ In a longitudinal follow up of 100 healthy infants born at term in the United States of America, 3% of 77 neonates were excreting virus, and 11% of 78 infants at 3 months.¹⁷ The prevalence at 6 months was 13% of 82 children, and at 1 year 11% had antibodies indicating past infection. At the examination at 2–2½ years no new excretors of CMV were found among the 30 susceptible children.

It has been suggested that acquired infection is more common in children from the lower socioeconomic groups; this is likely to be reflected by seropositivity rates, but in our study there was no association with social class when the serological state of the mother had been allowed for.

The rapid acquisition of CMV infection in infants of seropositive mothers within the first three months of life, and the relative absence of infection among infants of seronegative mothers, indicates that the mother is the major source of infection. Infection may be acquired during delivery, through contact with infected cervical secretions, or after birth. Important sources of postnatal infection include breast milk,^{11 18 19} and transfusion of seropositive blood products.^{20–22}

Reactivation of latent CMV infection and virus shedding from the cervix is common,^{23 24} and virus excretion may be enhanced during the immediate postpartum period.²⁵ There is poor correlation between cervical shedding during pregnancy and fetal infection, but the association between maternal shedding and transmission of infection to the offspring in early life is strong.¹¹ Reynolds *et al* reported the early acquisition of CMV infection in 40% of infants born to women from whom the virus had been isolated from the cervix in late pregnancy and concluded that CMV transmitted at the time of delivery was an important source of infection.²³ In a prospective study of 81 infants born to women known to be excreting CMV during pregnancy and in whom congenital CMV infection had been excluded, 21 (26%) had acquired infection by 1 year; 76% of these infections had occurred by 14 weeks.²⁶ In our study we had no information about cervical shedding of CMV, and virus was grown from the urine of only 1.2% of women.

Infected breast milk is an even more efficient source of acquired infection. Hayes *et al* isolated CMV from milk or colostrum, or both, from 17 of 63 (27%) seropositive women.¹⁸ Virus was isolated significantly more often after the first week

than before. Stagno *et al* reported the isolation of CMV at least once in breast milk or colostrum from 38 of 265 (14%) seropositive women: 25/70 (36%) in breast milk and 20/244 (8%) in colostrum.¹¹ In a population with a high prevalence of immunity among women of childbearing age transmission through breast milk is likely to be the major source of infection. Indeed, in the present study the acquisition of CMV among infants of seropositive mothers was significantly higher in the breast fed group than in the bottle fed group. Virus was isolated from the breast milk of 20% of seropositive mothers who breast fed. Surprisingly, however, despite the presence of CMV in breast milk four breast fed infants did not acquire CMV infection.

In a parallel study done in the same hospital, specimens of breast milk were taken from a random sample of 140 women seropositive for CMV in the first week after birth and from 137 women attending for their first postnatal examination at six weeks. Virus was isolated from 2.9% of the colostrum samples and 15.2% of the breast milk samples collected at six weeks. Virus excretion was not related to the mother's age, unlike the prevalence of virus shedding in urine and from the cervix, which diminishes with age.²⁷

Although CMV infection acquired in the neonatal period has been temporarily associated with pneumonitis,²⁸ it is seldom symptomatic, and we confirmed this. CMV infections transmitted by blood transfusion to premature infants born to seronegative mothers or those whose serological state was unknown, however, have resulted in severe and even fatal disease.^{20 21} It has been recommended, therefore, that blood products given to premature infants should be screened for CMV. It is not known whether expressed milk given to premature infants of seronegative mothers, who are the ones at greatest risk, may also become a source of serious nosocomial infection. It has been recommended that fresh donor milk fed to premature infants should be appropriately processed to reduce infectivity,²⁹ but this may decrease the potential benefit of the milk.

CMV infection is chronic, with viral shedding persisting for years. Our finding that children who had been infected in their first year still had virus in their urine at three years is important. This implies that infection in young children in the community is widespread and that most of this infection has been acquired from the mother at an earlier age. As acquired infection is so common in early life it is not possible to distinguish congenital from acquired infection after the first three or four weeks of life, even when neurological symptoms compatible with congenital infection are present.

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