*Original articles***Cytomegalovirus infection in day nurseries**

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SUMMARY One hundred and seventeen children and 41 teachers in day nurseries were screened for cytomegalovirus (CMV) viruria over a period of one year. Thirty two (27%) children and two (5%) teachers were found to be excreting virus on at least one occasion. Restriction endonuclease typing showed that virus strains isolated from the children were dissimilar, with the exception of those from sibling pairs and one unrelated pair. The virus isolate from one teacher matched those from two unrelated children, while the isolate from another teacher could not be distinguished from that from a sibling pair. The CMV serological state of the 41 teachers was not significantly different from 500 matched controls and no seroconversions occurred.

It is concluded that although transmission of CMV among children and teachers may occur in day nurseries, the dissimilarity of most of the virus strains indicates that infection predominantly occurs outside. Furthermore, teachers in day nurseries showed no evidence of an increased risk of past CMV infection when compared with matched controls.

Cytomegalovirus (CMV) infection acquired during pregnancy may cause fetal damage,¹ but when infection occurs postnatally the clinical consequences are usually trivial.² In the United Kingdom 3–4 per 1000 children are born with congenital CMV infection³ and a further 20% become infected during the first year of life.² The major source of early acquired infection is maternal breast milk.⁴

A non-maternal source of CMV infection thought to be important in the acquisition of CMV is group child care. In countries where most young children receive group care the rates of CMV infection are high in young age groups² and several studies of children attending day care centres have reported high rates of CMV excretion.^{5–9} With the exception of two studies,^{10 11} however, characterisation of CMV using deoxyribonucleic acid (DNA) restriction endonuclease analysis to show differences or similarities between virus isolates was not carried out, and without virus typing it can only be inferred that the reported high rates of excretion were due to transmission occurring within the centres. No studies have been performed in the UK. We report here the application of virus typing to CMV virus isolates obtained from children and teachers in day nurseries in south east England.

Methods

CMV excretion was studied in five day nurseries;

three were ordinary local health authority day nurseries, primarily serving local and disadvantaged children, and two specialised in the care of physically, emotionally, or intellectually handicapped children. In each centre children spent the entire day at the nursery. Classes were selected at random in three nurseries. One class in the fourth nursery was selected because a child with congenital CMV infection and known to be excreting the virus was enrolled. In the fifth, another nursery with a child with congenital CMV infection, all children participated in the study. Within each class children played together, shared toys, and ate lunch in a common room.

Informed consent was obtained from the parents of the study children. Parents also completed a questionnaire that provided data on demographic variables and day nursery experience. In four nurseries urine samples were collected from the children every three months for one year. In the remaining nursery two collections were made six months apart.

Urine specimens were collected using sterile U bags if the child was not toilet trained, and those children who were toilet trained voided into a sterile container or a clean receptacle. Specimens were refrigerated at 4°C until inoculation on to monolayers of human embryonic lung fibroblasts. All tissue cultures were incubated and maintained for four weeks.

Informed consent was also obtained from the teachers in the day nurseries and urine samples were collected on the same schedule as for the children. In addition, blood samples obtained from the staff at the beginning of the study and then at six monthly intervals were tested for the presence of CMV complement fixing antibodies. Serial samples on the same individual were tested simultaneously.

Restriction endonuclease analysis was carried out on all isolates in which there was sufficient growth to allow typing. The method for CMV differentiation by restriction enzyme nuclease analysis developed by Huang *et al*¹² and modified and simplified by Garrett and Warren¹³ was used. A minimum of three enzymes was used to cleave the 32P DNA prepared from each isolate, and the results of the typing were read blind.

The χ^2 test was used to test differences in sample proportions. When it was necessary to control for confounding variables the Mantel-Haenszel method for estimating odds ratio was used.¹⁴

Results

One hundred and seventeen children from the five day nurseries were included in the study. Characteristics of the study group are shown in Table 1. All the handicapped children were enrolled at the two special nurseries, which had significantly more white children than the others; 88% of the handicapped children were white compared with 38% of children in the other nurseries ($\chi^2=29.05$, $p<0.001$).

Overall, 32 (27%) children were found to be excreting CMV at some time during the study. Several characteristics were associated with increased risk of shedding virus (Table 2): significantly fewer white, older, and handicapped children were excreting CMV than other children.

Only one child attending a special nursery was excreting CMV. This child had congenital CMV and

Table 2 Characteristics of children excreting cytomegalovirus

	No (%) of children excreting cytomegalovirus (n=32)	p Value
Race:		
White	10 (16)	<0.01
Non-white	22 (40)	
Sex:		
Male	13 (22)	NS
Female	19 (33)	
Age (years):		
<2	12 (46)	<0.05
≥2	20 (25)	
Parents' social class:		
Non-manual	3 (15)	NS
Manual	11 (28)	
Unemployed	9 (36)	
Unknown	9 (31)	
Type of nursery:		
Special day nursery	1 (2)	<0.0001
Day nursery	31 (40)	

NS=Not significant.

had been in the nursery for nine months before the first screen. No other child in his class of 25 children was found to be shedding CMV at either of the two screenings six months apart. In the other nursery for handicapped children no children were found to be excreting CMV during the study.

In the remaining three day nurseries (A, B, and C) the prevalence of CMV excretion was high and 40% of children were found to be excreting CMV on at least one occasion. In nursery A the rate was 32% (9/28), in nursery B 46% (10/22), and in nursery C 50% (13/26). The number of children actually excreting virus at any one time ranged from 18% in nursery A to 37% in nursery C. Children excreting CMV tended to do so consistently throughout the study; 19% of the children excreting CMV did so for at least one year.

Neither the average age at which the child had entered the nursery nor the average time the child had been there was associated with excretion of the virus. Children excreting CMV entered the nursery at an average age of 16.5 months and had been in the nursery for an average of 15.5 months at the time of the first screen compared with 16.8 months and 16.2 months, respectively, for children who never excreted CMV.

Twenty (62%) of the 32 isolates were typed using restriction endonuclease techniques. The 12 isolates not typed were either lost due to technical problems or failed to yield sufficient growth for typing. Examples of similar and different oligonucleotide patterns have been described previously.¹⁵ In nursery A nine children were excreting CMV at some time during the study. Isolates from six

Table 1 Characteristics of children studied (n=117)

	No	(%)
Handicapped	41	(35)
Non-white	54	(47)
Sex:		
Male	59	(50)
Female	58	(50)
Age (years):		
<2	26	(22)
≥2	79	(78)
Parents' social class:		
Non-manual	24	(21)
Manual	39	(33)
Unemployed	25	(21)
Unknown	29	(25)
Toilet trained	69	(59)

children were typed and two, from siblings, were found to have indistinguishable DNA patterns. Ten children were excreting CMV at some time in nursery B and isolates from nine were typed. Again two siblings excreted virus with indistinguishable oligonucleotide patterns as did two unrelated children. All the isolates were dissimilar to that obtained from the child with congenital CMV. In nursery C it was only possible to type isolates from five of the 13 children excreting CMV, of which three were dissimilar and those from two siblings could not be distinguished. Overall, therefore, two unrelated children attending the same nursery and three sibling pairs shed virus that could not be distinguished.

Forty one teachers were employed in the five day nurseries. Their ages ranged from 19 to 57 years, with a mean of 32.4. Eighty eight per cent were white. To determine whether the teachers were at increased risk of acquiring CMV, the prevalence of CMV antibody state in the teachers was compared with that of 500 women in their first pregnancy, matched for race and social class, attending a maternity unit in the same area. As the mean (SD) age of the teachers (32.4 (11.9) years) was significantly higher than that of the controls (29.53 (4.46) years) ($t=3.29$, $p<0.001$) the Mantel-Haenszel method was used to determine whether there was a significant difference in CMV antibody state between the groups. CMV seropositivity was found in 66% of the teachers compared with 53% of the controls. The adjusted odds ratio (1.67) for these two rates was not significant ($\chi^2=1.47$, 95% confidence limits 0.739–3.6).

None of the 14 seronegative teachers seroconverted during the study, but two teachers, both from the same nursery, did excrete virus. The oligonucleotide patterns of virus isolates obtained from both teachers were compared with each other and with those from children in the nursery. The DNA patterns of isolates from the two teachers were dissimilar. The pattern of the isolate from one of the teachers could not be distinguished, however, from the DNA pattern of isolates from a sibling pair. The other teacher excreted virus that could not be distinguished from that isolated from another child in the nursery. As the teachers were both seropositive at the beginning of the study the direction of transmission—child to teacher or teacher to child—could not be determined.

Discussion

The present study confirms previous reports of a high prevalence of CMV excretion in young children attending day nurseries.⁶⁻⁹ The application of re-

striction enzyme typing of viral DNA allows transmission pathways to be identified with more precision, although in the present study a substantial proportion of the virus strains could not be typed owing to difficulties in growing sufficient quantities of the virus. The dissimilarity between strains from all but two children other than siblings suggests that most children acquired their infection outside the nursery. Although virus strains were not dissimilar in two unrelated children, suggesting horizontal transmission of infection, this may reflect an extraordinary chance event. In a parallel family study in which an infant acquired infection after a blood transfusion¹⁵ a strain indistinguishable from those isolated from the above two nursery children was identified; epidemiological investigation failed to identify any common source.

Two previous studies in day nurseries have used the restriction enzyme technique, but no teachers were included.^{10 11} Adler studied one nursery of 66 children where 16 were excreting CMV.¹⁰ Seven children under 29 months were excreting CMV strains that had indistinguishable DNA patterns. Four other children were excreting a virus different from the first group but indistinguishable from each other. Two day care centres were studied in Sweden.¹¹ In one, seven of 20 children were excreting CMV, three had indistinguishable DNA patterns, and four were unique. In the other nursery five of 16 children were excreting CMV and all strains were unique.

In a largely white middle class population in west London as many as 20% of children acquired CMV by the age of 1 year and continued to shed virus for up to three years. (Peckham C. Unpublished observations.) One third of the children whose mothers were seropositive, however, were excreting CMV by their first birthday. Forty per cent of the children in our day nursery study were non-white, a population known to have a higher seropositive rate. Many of the children would probably have been excreting virus, therefore, after infection acquired from their mothers and before entering the day nursery.

Data from previous day care studies in the United States suggest that virus transmission between children may occur within day centres. In one study 51% of children were excreting CMV, with the highest rate in children under 24 months (83%);⁶ at least 12% of these children had seronegative mothers. In another study rates of CMV excretion were significantly higher for children receiving day care compared with children staying at home.⁷ Only 8% of the children at home had viraemia compared with 57% of children in day care, but without restriction enzyme analysis it is not possible to draw any firm conclusions. Two case reports have de-

scribed situations where transmission of CMV was assumed to have occurred but when restriction endonuclease typing was carried out this was found not to have been the case.^{16 17}

Our study points to the risk of generalising from only one or two nurseries. The wide variation in the rates of CMV excretion (0% to 36%) in our nurseries indicates that not all day care settings are the same. We were asked to include two nurseries in the study because two children known to have congenital infection were considered to be a potential source of infection for other children and staff. In both situations there was no evidence that this was the case and in the nursery for handicapped children no other child was found to be shedding CMV.

The risk of CMV infection for teachers in day care centres cannot be established from our data as numbers are too small. Although in two cases we were unable to distinguish virus isolates from children and teachers, the seropositivity rate for teachers was not significantly different from controls and no seroconversion occurred. Further prospective studies are required to determine whether the risk of infection among day nursery teachers is different from that of women of similar age and background in the general population.

Although the findings suggest that transmission of infection may occur within some nurseries, this does not seem to be the case for most infections. CMV infection is commonly acquired in the first year of life, particularly among infants of seropositive mothers, and virus shedding persists for a prolonged period. It is not surprising, therefore, that virus excretion is common among young children attending day nurseries. In view of the fact that virus shedding was more prevalent in the younger than the older children and was not related to the age at which the children entered the nursery nor to the duration they had been there and the dissimilarity of most of the virus isolates, the present study indicates that most infection probably occurred before entering the nursery.

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