

Seroepidemiology of cytomegalovirus infections during the first years of life in urban communities

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SUMMARY Using indirect haemagglutination assay, combined with a collection of blood samples on blotting cards, seroepidemiological surveys of cytomegalovirus infections during early infancy have been done in different populations (French and immigrant) in urban areas. The comparison of CMV antibody status of mothers and their children at 10 months and at 2 years of age enables possible factors of viral transmission to be defined. During the first year of life, seropositive mothers were the only source of infection and they remained the main source during the second year. Socioeconomic class and educational level are determinant factors in the incidence of viral transmission.

There are wide variations in the incidence of infection by cytomegalovirus (CMV) in infants, related to socioeconomic classes and ethnic groups (Lang, 1975; Hanshaw, 1976). Epidemiological analysis of these variations is needed but this requires large surveys in different populations. This has been difficult because virus isolations cannot be used in large surveys, and serological tests have been made mainly by complement fixation (CF), a test having variable sensitivity; some CF-negative subjects had detectable CMV specific antibodies when more sensitive techniques (immunofluorescence, platelet-agglutination) were used (Weller, 1971; Stagno *et al.*, 1975).

The indirect haemagglutination (IHA) assay (Fucillo *et al.*, 1971; Huraux *et al.*, 1971) is a sensitive and specific technique for detecting CMV antibody and it can be used on a large scale for seroepidemiological surveys, especially when it is combined with the collection of blood samples on blotting cards requiring only a few drops of blood (Cabau *et al.*, 1976). We used this technique in a seroepidemiological

study of CMV infections on infants during the first two years of life in different urban populations.

Subjects and methods

This study was carried out at two public health centres where children are seen for health check-ups at ages 10 months, 2 years, and 4 years. Children and their mothers spend between 3 and 4 hours in the centre during which time the child has a complete medical examination and some laboratory tests, and the mother has a psychological interview. The first centre (centre A) is in Clichy, a suburb north of Paris in which socioeconomic conditions are generally low and housing facilities poor. The second (centre B) is in the 13th district of Paris (south); a large part of it has recently been rebuilt with modern apartments, and it is inhabited mainly by the middle class. In both centres about 80% of people are French, the others are immigrants mainly from Africa, Spain, or Portugal. A blood sample was taken from the finger of mother and child, and sometimes from the father, filling the three circles of the blotting cards for a PKU test.

Information about the socioeconomic conditions of the families was obtained and clinical investigations were made by nurses and paediatricians not connected with the study. Once a week the cards were posted to the laboratory.

Serology. On arrival at the laboratory the cards were stored at 4°C. Serum was eluted from the dried blood specimens collected on the three circles in 1 ml buffer, giving a dilution of about 1:10 of the serum.

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Serum was eluted within two weeks of taking the blood specimen. IHA assays were used for CMV antibody with the AD-169 strain of CMV as source of antigen, using the technique of Fucillo *et al.* (1971) modified by Cabau *et al.* (1976). Each sample was titrated from 1:10 up to 1:5120; mothers with an antibody titre <1:10 and 10-month-old children with an antibody titre <1:40 were considered negative. A previous longitudinal serological study had shown that in some infants born to CMV antibody-positive mothers, maternally-transmitted CMV antibodies could still be detected at 10 months by IHA assay, but the titres were <1:40 (Cabau *et al.*, 1977). After a primary CMV infection proved by the presence of CMV antibody in IgM fractions, antibody titres were >1:40 at 10 months in every case; so in this study any infant with a CMV antibody titre >1:40 was presumed to be infected. It is not possible to establish if some of these infections were acquired *in utero*.

Results

CMV antibody studies in mothers and their 10-month-old children. 229 mothers and their children were tested at centre A and 399 at centre B (Table 1). At

centre A, 71.6% of the mothers had CMV antibody compared with 56% at centre B. In the French mothers, 66.5% of them gave seropositive results at centre A compared with only 47.4% at centre B. At both centres a high percentage (about 90%) of immigrant mothers had CMV antibodies.

Comparison of CMV antibody in the 10-month-old infants shows striking differences: in centre A, 28.4% were seropositive; in centre B, 10%. Among French children 25.8% were seropositive in centre A, and 5.1% in centre B. A small difference was noted among immigrant children: 38% in centre A, 27% in centre B.

All 10-month-old infants who were seropositive had seropositive mothers, while none of the seronegative mothers had a seropositive child. Among seropositive mothers, in centre A 47 (39%) of 121 French mothers and 18 (42%) of 43 immigrant mothers had a seropositive child: in centre B 16 (11%) of 147 French mothers, and 24 (31%) of 77 immigrant mothers had a seropositive child. The serological titres of mothers were not related to the fact of whether or not their children were seropositive.

Some factors which may affect the transmission of virus from mother to infant were studied. There were no differences in the proportion of children delivered by caesarean section, nor were there differences in the proportion of children who attended day nurseries during the first months of life.

The proportion of children who were breast fed by seropositive mothers was studied in relation to the antibody status of the child at 10 months of age (Table 2). When results of the two centres were combined, significant differences (but only at the 5% level) were found between seropositive children (29% were breast fed <8 weeks and 23% >8 weeks) and seronegative children (20% <8 weeks and 15% >8 weeks).

At both centres certain socioeconomic factors (housing, education) were correlated with CMV antibody status of the mother and the child. In centre A there were no differences as all these people were in lower socioeconomic classes. In centre B some significant differences emerged: if both mother

Table 1 *CMV antibody status of mothers and 10-month old children*

Centre	Population		CMV antibody status	
	Ethnic groups	No.	Positive mothers No. (%)	Positive children No. (%)
A	French	182 (79.5%)	121 (66.5)	47 (25.8)
	Immigrant	27	26 (96)	10 (38)
	African	16	14 (87)	5 (35)
	Spanish-Portuguese	4	3	3
	Others	229	164 (71.6)	65 (28.4)
B	French	310 (77.7%)	147 (47.4)	16 (5.1)
	Immigrant	45	37 (82)	11 (24)
	African	16	15 (94)	3 (19)
	Spanish-Portuguese	28	25 (89)	10 (35)
	Others	399	224 (56)	40 (10)

Table 2 *Antibody status of the child related to breast feeding*

	Centre	Mother + Child+	Mother + Child-	Significance of difference	Mother - Child-
Breast feeding < 8 weeks	A	27/99	14/65	NS	16/61
	B	15/46	34/178	S	38/178
	Total	42/145 (29%)	48/243 (20%)	S	54/239 (23%)
Breast feeding > 8 weeks	A	23/99	7/65	S	10/61
	B	11/46	29/178	NS	23/178
	Total	34/145 (23%)	36/243 (15%)	S	33/239 (14%)

NS = Difference between the groups mother+child+, mother+child- is not significant; S = significant at 5% level between the groups: mother+ child+, and mother+child-.

Table 3 Socioeconomic conditions in centre B families

Criteria	CMV antibody status		
	Mother+ Child+	Mother+ Child-	Mother- Child-
Families with good housing standards (%)	39	46	59
Fathers with only elementary education			
Total	27	12	13
French	35	9	8.7

and child were seropositive their socioeconomic conditions tended to be low (Table 3).

The educational level of the fathers was compared. In French families, for which information was more reliable, in cases where both mother and child were seropositive, only 35% of fathers had had elementary education alone, compared with 9% of fathers in the other two groups.

Development of CMV antibodies in children at 10 months and 2 years and in their mothers. At centre B we collected blood samples from 262 mothers and their children at a second check-up examination at 2 years of age. Table 4 shows correlations between the results for each mother and child according to the antibody status of the 1st and 2nd samples. In 247 (94.3%), no change was observed in the CMV antibody status of mother and child. Nine CMV seronegative 10-month-old children with seropositive mothers became seropositive at 2 years of age. Two CMV negative 10-month-old children became seropositive but their mothers remained seronegative. Four seronegative mothers became seropositive and, in two instances, the child also became seropositive.

Differences were found between the French and immigrant families. In the 224 French families, 13 (6%) had a seropositive child at 10 months and 20 (9%) at 2 years. If the French seropositive mothers are considered, 13 (13%) out of 97 had a 10-month-old seropositive child and 18 (18%) out of 100 a seropositive child at 2 years. In the 38 immigrant families 12 (31%) had a seropositive child at 10 months and 18 (47%) at 2 years. In the group of seropositive mothers the figures are respectively 12 (34%) out of 35 and 18 (50%) out of 36.

Among factors which may indicate a possible

source of CMV infection we noticed that in the 9 children who had seropositive mothers at 10 months and who became seropositive at 2 years, 6 stayed in day nurseries and 3 were nursed at home by their mothers. The 2 children who became seropositive with their mothers still seronegative, and the 2 children who seroconverted, as did their mothers, attended day nurseries from age 4-5 months. In the two observations in which the mothers became positive, only in the 2nd sample was the child nursed by the mother.

As a result of the clinical examination of the infants at 10 months and at 2 years, nothing of note was detected in those children with CMV antibodies.

Discussion

The use of IHA assays of blood samples collected on blotting cards is practical for surveys such as this. Samples may be posted, as an earlier study (Cabau *et al.*, 1976) showed that if elution is done within 2 weeks, correlation with titres using conventional serum samples is good. Even in specimens with low titres, no false-negatives were found. The method is useful for large surveys, and is accepted readily by the population (especially in infants from whom it is easy to obtain a few drops of blood) and by the doctors and nurses who collect the samples.

The presence of CMV antibodies in a mother at the time of the visit of her 10-month-old child probably reflects her antibody status at pregnancy. Of the 262 women who were tested twice at an interval of 14 months, only 4 showed seroconversion. Of the mothers who were seropositive in both tests, none showed significant change in titre. This stability of CMV antibody titre using IHA assay contrasts with fluctuations of CF antibody titre (Waner and Weller, 1973).

In this study the presence of CMV antibody after a primary infection in the child could clearly be seen to relate to the presence of CMV antibody in the mother, consistent with earlier reports (Levinsohn *et al.*, 1969; Andersen *et al.*, 1972; Leinikki *et al.*, 1972; Granström *et al.*, 1977).

Intrapartum contact with infected cervical secretions has been supposed to be the main cause of these

Table 4 CMV antibody status of mothers (M) and their children (C) at 10 months and 2 years of age

	CMV antibody status						
	No change			Change			
10 months	M+C+	M+C-	M-C-	M+C-	M-C-	M-C-	M-C-
2 years	M+C+	M+C-	M-C-	M+C+	M+C-	M+C+	M-C+
French	13	80	122	4	2	1	2
Immigrant	12	18	2	5	0	1	0
Total	25	98	124	9	2	2	2

early infections. A longitudinal study on some children without specific IgM antibody at birth, but in whom CMV antibody was present at one year, showed that they had all acquired detectable specific IgM antibody between 3 and 6 months of age, suggesting early contamination, probably an intrapartum viral transmission (Cabau *et al.*, 1977). The fact that the incidence of caesarean sections did not vary in the different groups may be due to the fact that it was not possible to determine whether caesarean sections were before or after membrane rupture.

The percentage of children who were breast fed is significantly higher (but only at 5% level) in the group of children who had a primary CMV infection. When these results are correlated with socioeconomic criteria (e.g. parents with primary educational standard only) the difference remains. The high frequency of breast feeding is not related to low socioeconomic conditions, in agreement with general observations in French urban populations.

Consideration of the 13 children who showed CMV seroconversion between 10 months and 2 years supports the idea that mothers are the most important source of infection (9/13). Other sources may exist; for instance, both children who acquired CMV antibodies while their mothers remained negative attended nurseries. In the 2 cases where mothers and children both became CMV seropositive, the children also attended day nurseries and it is not known whether the mother or the child was first infected.

We conclude that during the first year of life mothers constitute the main, if not the only, source of CMV infection, and they remain the most common source of infection during the 2nd year of life also. In seropositive mothers CMV has been isolated in cervical secretions and in milk (Hayes *et al.*, 1972), and CMV infection is also considered a 'kissing disease'. During the 2nd year of life some extra-maternal sources of infection appear. Close contact between children in day nurseries is a possible mode of spread, but seems rare: 47% of seronegative children with seronegative mothers attended day nurseries, but no more than 4 children were infected by CMV.

This survey also demonstrates the importance of socioeconomic conditions in the transmission of CMV infections during the first 2 years of life. The incidence of infection depends on two factors: (1) the percentage of seropositive mothers, the potential source of virus, and (2) the percentage of infection of children from seropositive mothers.

In our groups of differing socioeconomic class, the percentage of seropositive mothers differed (centre A 66.5%; centre B 47.4%) as did the frequency of infection in their children (centre A

38.8%; centre B 10.8%). These two factors result in figures of 5 to 25% for the acquisition of seropositivity by 10 months in the two groups. In other surveys on French infants we have found a figure of 5% in a rural group and 76% in a poor urban group (Boué *et al.*, 1976, and unpublished).

In immigrant groups, the percentage of seropositive mothers was about 90%. They were infected mainly in their native country, but a slight difference is shown in the percentage of their seropositive children (centre A 41.8%; centre B 31.1%). In a previous study of very poor immigrant families living in Paris suburbs, 90% of the infants were seropositive (Boué *et al.*, 1976), a high frequency of early acquisition of antibody and similar to that observed in African populations (Lang, 1975; Cabau *et al.*, 1977). The improvement in living and educational standards of immigrant families reduces the frequency of early infection. It is of note that in centre A where the standard of living was similar in both French and immigrant groups, the percentages of seropositive children born from seropositive mothers were also very similar. In contrast with the above figures, only about 5% of 'middle-class' 10-month-old children have CMV antibodies.

These facts underline the importance of the standard of living in determining the transmission rates of CMV infections from mothers to infants. Not only are economic conditions (housing, income of the family) relevant, but so also are the prevailing hygiene habits, and these can be influenced by education.

We have found that the use of IHA assays of blood collected on blotting cards provides a practical tool for large surveys. What is needed is to follow-up pregnant women and their babies in different population groups, and then measure the incidence of intrauterine infections and their relation to living standards and personal hygiene. Such studies are indispensable if policies leading to prevention are to be formulated.

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