
Article

Role of Cytomegalovirus Infection in the Incidence of Viral Acute Respiratory Infections in Children Attending Day-Care Centers

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Abstract In a prospective study carried out in Lyon, France, the association between the excretion of cytomegalovirus (CMV) and the increasing frequency and severity of viral respiratory infections in children attending day-care centers was evaluated. Urine samples were collected in November 1992 (S1) and 4 months later in February 1993 (S4). A total of 246 children aged 6–12 months attending 29 day-care centers from 1 November to 28 February were screened for the excretion of CMV in urine. The diagnosis of viral acute respiratory infection was performed in the case of outbreaks only. Forty-eight (19.5%) children were both S1 and S4 positive for CMV, 30 (12.4%) became CMV positive (S1–/S4+), 4 (1.6%) became negative (S1+/S4–) and 164 (66.7%) remained negative. The percentage of children becoming CMV positive was significantly ($P < 0.001$) higher in day-care centers where more than 40 children were enrolled. Nine outbreaks due to respiratory syncytial virus, rhinovirus and enterovirus were recorded in 8 of 29 (27.6%) day-care centers. Viral acute respiratory infections were significantly ($P < 0.05$) more frequently recorded in day-care centers in which CMV and respiratory viruses cocirculated and were significantly ($P < 0.001$) more frequently reported in CMV-infected children. These findings suggest that viral acute respiratory infections are significantly more likely to occur in CMV-infected children.

Introduction

Cytomegalovirus (CMV)-excreting children attending day-care centers have long been considered a major source of CMV transmission to seronegative children and women, including day-care providers and mothers of childbearing age [1, 2]. CMV transmission from child to child [3, 4], child to parents [3, 5, 6] and child to day-

care providers [4, 7–10] has been studied extensively. For example, an annual CMV infection rate of 10–20% has been reported in CMV-seronegative women working in day-care centers in the USA [8]. A similar study conducted in Cedar Rapids, Iowa (USA), has estimated the overall risk to day-care providers of contracting CMV infection from infected children attending day-care centers to be 22% and 40% for a 12- and 16-month exposure period, respectively [11]. In France, a study of CMV transmission in 93 children attending six day-care centers in Val de Marne (near Paris) showed that, over a 6-month period of surveillance, 23.4% of 6–12-month-old children excreted CMV. In this study, the phylogenetic analysis of the strains recovered revealed no dominant or more efficiently transmitted strain [12].

Besides CMV infection, viruses responsible for respiratory diseases spread every winter and may be responsible for epidemics in day-care centers [6, 13]. In a previous study conducted in Lyon over four winter

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seasons (1988-99, 1989-90, 1992-93, 1993-94), 83 outbreaks due to respiratory viruses in 47 day-care facilities were recorded. During this study, 36% of the children attending day-care centers were infected by respiratory viruses, including respiratory syncytial virus (RSV), influenza viruses A and B, parainfluenza virus, coronavirus, rhinovirus, adenovirus and enterovirus. The respective prevalence of each virus was different each year, with RSV causing about one-third of the respiratory tract infections reported [14].

As CMV has been found to cause immunosuppression [15] and to increase the severity of RSV infection [16], we evaluated the rate of CMV infection among children 6–12 months of age attending day-care centers and estimated the impact of CMV infection on the occurrence of viral acute respiratory infections, especially those due to RSV, by monitoring the incidence of viral acute respiratory infections (ARIs) in the study group.

Materials and Methods

Participants. During a study implemented in day-care centers in Lyon, France, from November 1992 to February 1993, data obtained from the service Santé-Prévention of the “Conseil Général du Rhône” showed that 4,186 children attended 198 day-care centers. A surveillance study for CMV infection and viral ARIs was carried out in 29 of the 198 (14.6%) day-care centers, representing a population of 246 of 4,186 (5.9%) children 6–12 months of age attending day-care centers. Approximately 80% of all children attending day-care centers in Lyon belong to this 6–12-month age group.

The children were divided into six groups by age (6–7 months, 7–8 months, 8–9 months, 9–10 months, 10–11 months and 11–12 months). Furthermore, day-care centers were divided into four groups (10–20, 21–30, 31–40 and over 40 children enrolled), as shown in Table 1. All children included in our study attended the centers full-time, spending 8–10 hours per day, 5 days per week in day-care facilities. We had no information on the siblings.

Samples. Urine samples were collected during November 1992 (S1) and then 4 months later, during February 1993 (S4). Surveillance for ARIs was implemented in the 29 day-care centers during the same period only in the event of an outbreak. An outbreak was recorded when at least four children or 25% of the children attending a given day-care center presented with similar ARI signs and symptoms within a period of 3 days. When an ARI outbreak was recorded, nasal swabs were collected from all the children attending the facility, including those that remained home due to illness.

Detection of Cytomegalovirus by Culture. S1 and S4 urine specimens were collected in sterile individual bags (Urino-col-Biotrol, France). The sealed bags were hand-carried to the laboratory within 1 h at 4°C and processed immediately. Urine samples were filtered through a 0.45-micron membrane (Millipore, France) to eliminate bacterial and fungal contamination. Two hundred microliters of the filtrate was inoculated by low-speed centrifugation at 700 × g for 45 min at 37°C onto MRC5 human diploid cells, as the centrifuged inoculum proved to be more sensitive for CMV isolation [17]. Each specimen was inoculated in duplicate onto two different plates and incubated at 37°C with 5% CO₂. MRC5 cells of one plate were fixed after 48 h of culture for the detection of CMV immediate early antigens, the second plate

being used to detect CMV through cytopathic effect. Plates were checked daily for 4 weeks to detect cytopathic effect.

Detection of Cytomegalovirus Immediate Early Antigens. This test has been designed for the rapid (48 h) and sensitive detection of CMV immediate early antigens [17]. We amplified the detection signals by using an avidin-biotin reaction [18]. After urine sample inoculation, the monolayer was incubated for 48 h prior to being fixed for 10 min at 4°C with methanol (Merck, France). The cells were then washed three times with pH 7.2 phosphate-buffered saline (PBS) (Merck). One hundred microliters of a 1:150 dilution in PBS of the mouse monoclonal antibody E13 (Biosoft-Argene, France), specific for an epitope of the major immediate early gene, was added to the fixed monolayer and incubated for 30 min at 37°C. Then, the cells were washed three times with PBS and 100 µl of a 1:100 PBS diluted biotin-labeled goat anti-mouse antibody (Biosoft-Argene) was added to each well and incubated for 30 min at 37°C. The cells were washed three times in PBS, and 100 µl of PBS-diluted (1:600) avidin-peroxidase conjugate (Biosoft-Argene) was added and incubated for 15 min at 37°C. Peroxidase was subsequently revealed for 10 min at 25°C with 100 µl of 2, 2' diaminobenzidine tetrahydrochloride substrate (Sigma, France) dissolved in distilled water at a 1 mg/ml concentration. Intranuclear brown inclusions specific for immediate early antigens were observed under a light microscope (magnification ×400). Each test included two negative controls (MRC5 cells without inoculum) and two positive controls (AD169 strain, ATCC, Rockville, MD, USA) at a calibrated dilution giving at least 10 positive nuclei per positive control well.

Detection of Respiratory Viruses During Outbreaks. Concomitantly, the 29 day-care centers were monitored for outbreaks of viral respiratory infection. Detection of respiratory virus from nasopharyngeal cells was performed as described previously by conventional immunofluorescence staining using specific monoclonal antibodies to detect RSV antigens (Biosoft-Argene) and by an immunocapture EIA test to detect influenza A and B and parainfluenza virus antigens, as described previously for influenza [19]. The samples were also inoculated onto cell cultures (MRC5, MDCK, Hep2) [20, 21] and checked daily for cytopathic effect of rhinovirus, enterovirus, adenovirus, influenza, parainfluenza and RSV. The viruses isolated were identified using conventional procedures [21].

Statistical Analysis. As a probability test, the chi-square test was applied to each statistical evaluation.

Results

Sampling of Children for Detection of Cytomegalovirus and Viral Acute Respiratory Tract Infection. Eight to nine children per day-care center were sampled for diagnosis of both CMV infection and viral ARI. No significant difference ($0.05 < P < 0.1$) was observed when the smallest number of children attending a single day-care center ($n = 53$) was compared with the highest number of children attending a single day-care center ($n = 69$). The number of children per age group was equivalent, ranging from 37 to 45. The female-to-male sex ratio of children was not significantly different among the day-care centers ranging from 1.1 to 1.3, with a mean value of 1.22.

Caretakers and Hygiene in Day-Care Centers. Regardless of the size of the day-care center, the ratio of care-

Table 1 Influence of day-care center size on cytomegalovirus (CMV) shedding in urine. The number of children attending each group of day-care centers (DCCs) is not significantly different

Size of DCC, according to no. of children enrolled	Total no. of children attending	No. of children per group (group 1–group 4) ^a			
		Group 1	Group 2	Group 3	Group 4
10–20 enrolled (<i>n</i> =7 DCCs)	58	11	4	1	42
21–30 enrolled (<i>n</i> =8 DCCs)	53	10	4	1	38
31–40 enrolled (<i>n</i> =8 DCCs)	66	14	4	1	47
>40 enrolled (<i>n</i> =6 DCCs)	69	13	18	1	37
Total (<i>n</i> =29 DCCs)	246	48	30	4	164
Percent	100	19.5	12.4	1.6	66.7

^a Group 1, children who excreted CMV during the 4-month period of surveillance; group 2, children who became CMV excretors; group 3, children who became CMV nonexcretors; group 4, children who did not excrete CMV

takers to children was identical (1:5). On interviewing the staff of each day-care center, no differences were noted in the workers' skills or the hygiene of the day-care centers. The number of toys was equal to the number of children, and the ratio of toys to children did not vary significantly (1–2 toys, with a mean value of 1.37; data obtained from the directors of 15 of 29 day-care centers of various sizes).

Follow-up of Cytomegalovirus Infection. During the 4-month period of surveillance for CMV infection, 48 of 246 (19.5%) children had CMV in both urine samples (group 1: S1+, S4+), 30 of 246 (12.2%) became positive (group 2: S1–, S4+), 4 of 246 (1.6%) became negative (group 3: S1+, S4–) and 164 of 246 (66.7%) remained CMV negative (group 4: S1–, S4–). Overall, the S1 urine sample was positive in 52 of 246 (21.3%) children at study entry, compared with 78 of 246 (31.7%) children who acquired CMV infection 4 months later; these figures were significantly different ($P < 0.01$). Day-care facilities were divided into four groups, according to their size (10–20, 20–30, 31–40 and over 40 children). As shown in Table 1, the number of children attending day-care centers, when divided into four groups (10–20, 21–30, 31–40, over 40 children), was not significantly different ($0.05 < P < 0.1$), whereas the number of children who became positive for CMV (group 2) was significantly greater in day-care centers that enrolled over 40 children (18 vs. 4 in each other group of smaller day-care centers, $P < 0.001$). During the surveillance, physicians responsible for each day-care center did not observe obvious clinical symptoms that might be associated with CMV infection, except in one 8-month-old child from group 1 who presented with adenopathy and fever and recovered within 1 month.

Follow-up of Viral Acute Respiratory Infections. According to outbreak criteria, nine viral ARI outbreaks occurred in 8 of the 29 (27%) day-care centers included in the study (Table 2). The viruses responsible for these epidemics were RSV (*n*=5), echovirus 11 (*n*=2) and rhinovirus (*n*=2). In one day-

($0.05 < P < 0.1$), whereas the number of children becoming CMV excretors in urine is significantly greater ($P < 0.001$) in large DCCs enrolling over 40 children

Table 2 Distribution of nine outbreaks of viral acute respiratory infection observed in eight day-care centers (DCCs) in Lyon

No. of children per DCC	No. of DCCs per group	Viral etiology (no. of episodes)	Month of outbreak (no. of episodes)
10–20	7	echovirus type 11 (1)	November (1)
21–30	8	rhinovirus (1)	November (1)
31–40	8	rhinovirus (1)	February (1)
>40	6	RSV (2)	December (2)
		^a RSV (1) and echovirus type 11 (1)	December (2)
		RSV (2)	February (2)
Total	29	9	9

^a Overlapping outbreaks in one DCC
RSV, respiratory syncytial virus

care center, two ARI outbreaks (RSV and echovirus 11) were overlapping. As shown in Table 2, six epidemics were recorded in day-care centers in which more than 40 children were enrolled (5 due to RSV and 1 due to echovirus type 11), whereas the three remaining epidemics were recorded in smaller day-care centers (1 due to echovirus 11 and 2 to rhinovirus). All the outbreaks were recorded between November 1992 and February 1993.

Influence of Cytomegalovirus on Viral Acute Respiratory Infection. As shown in Table 3, in 8 of 29 (27.6%)

Table 3 Spread of cytomegalovirus (CMV) and respiratory viruses in 29 day-care centers (DCCs) in Lyon. Viral outbreaks were significantly ($P < 0.04$) more frequently recorded in DCCs where CMV cocirculated

No. of DCCs	No. of DCCs		Total
	Viral ARI positive	Viral ARI negative	
CMV positive	8	1	9
CMV negative	0	20	20
Total	8	21	29

ARI, acute respiratory infection

Table 4 Influence of cytomegalovirus (CMV) infection on the incidence of viral acute respiratory infections (ARIs). Viral ARI and CMV infection are strongly linked (correlation coefficient=0.93)

	CMV infection (no. of children)		Total
	Positive	Negative	
Viral ARI (no. of children)			
Positive	21	2	23
Negative	3	46	49
Total	24	48	72

day-care centers, CMV and respiratory viruses were both detected, in one (3.4%) day-care center CMV alone was detected, and in 20 (69%) day-care centers no viruses were found. Viral epidemics were significantly ($P<0.04$) more frequently recorded in day-care centers where CMV cocirculated.

As observed in Table 4, among the 72 children sampled for viral ARI, 24 (33.3%) were shedding CMV and 48 (66.7%) were not. Among the 23 children positive for respiratory viruses, 21 (93.9%) were shedding CMV and 2 were not. Twenty-one of the 24 (87.5%) CMV-shedding children presented with viral ARI, compared with 3 of 24 (12.5%) who did not ($P<0.001$), and 46 of 48 (95.8%) CMV-negative children did not have viral ARI, compared with 2 of 48 (4.2%) children who had viral ARI ($P<0.001$). The two infectious events were strongly correlated (correlation coefficient=0.93). As shown in Table 5, children positive for both CMV and viral ARI were significantly more frequently infected with RSV than with other viruses (15/21 [71.4%] vs. 6/21 [28.6%]; $P<0.01$), and children who became CMV excretors during the study had significantly more RSV infections than children who excreted CMV at study entry (14/15 [93.3%] vs. 1/15 [6.7%]; $P<0.001$). RSV infections (bronchiolitis and rhinopharyngitis) were significantly more frequent in 6–8-month-old children (14/15 [93.3%] vs. 1/15 [6.7%]; $P<0.001$), and RSV-positive children had bronchiolitis more frequently (13/15 [86.7%] vs. 2/15 [13.3%]; $P<0.001$). Clinically, one

Table 5 Age distribution of children with both cytomegalovirus (CMV) infection and viral acute respiratory infection (ARI). Respiratory syncytial virus (RSV) infections were more frequently observed in children <8 months old, and children who

Viral etiology (symptoms)	No. of children in each age group			
	6–7 mos.	7–8 mos.	8–9 mos.	9–10 mos.
Rhinovirus (rhinitis)		2		
Echovirus type 11 (rhinopharyngitis)		1	1	2
RSV (bronchiolitis, pharyngitis)	10 ^a (group3)	3 ^a +1 ^b (group 3)	1 ^b (group 1)	

^a Bronchiolitis

^b Pharyngitis

7-month-old child who was a CMV excretor at the start of the study presented with serious bronchiolitis. He was hospitalized for 8 days, then recovered.

Discussion

In very young children, CMV shedding in urine has been shown to last for several months after CMV infection [22, 23]. We decided, therefore, to screen for CMV infection using urine samples from 246 children attending day-care centers in Lyon. However, we cannot rule out the possibility that a previous CMV infection was just reactivated in some children. Because of failure to obtain parental consent, we did not collect blood samples, which would have helped to differentiate primary infections from reactivations.

A total of 47 (20.8%) children had CMV in their first urine sample. This result was consistent with previously published data [4, 10, 11]. In the second urine sample, we observed a significant increase in the number of CMV-excreting children, consistent with the results (31.4% vs. 21.4%, respectively) observed in a study implemented in Paris [12], but much greater (31.4% vs. 9%, respectively) than the mean percentage observed in 1986 in the USA in children of the same age group [3]. The increasing percentage of children attending day-care centers in Lyon (in recent years, an increase of about 40% has been observed) may explain this difference. The percentage of CMV-excreting children was significantly ($P<0.001$) greater in large day-care centers (>40 children enrolled). This result was in agreement with those observed in a study conducted in 1991 in the USA [5]; the authors suggested that the number of children per day-care center was the main factor that affected rates of CMV infection. As observed previously [22, 23], all but one child presenting with CMV shedding were asymptomatic.

CMV transmission is linked to its ability to remain infectious on surfaces and toys for at least 8 h and in wet diapers for at least 12 h [24]. Since the source of transmission is the infected child, prevention of trans-

became CMV excretors during the study had significantly more RSV infections. No coinfections were observed in children >10 months of age

Group 1, children who excreted CMV during the 4-month period of surveillance; group 3, children who became negative for CMV

mission requires (i) periodic disinfection of surfaces and toys, (ii) rapid elimination of potentially contaminated wet diapers, (iii) frequent hand-washing and (iv) the use of gloves when handling potentially infected diapers, as suggested previously [4, 10, 25]. During our survey, steps (i) plus (ii) plus (iii) were put into practice in each day-care center, but gloves were not systematically used by day-care providers (except with children presenting with diarrhea), especially in large day-care centers. Given that (i) no significant differences were observed regarding the number and the age of children attending the various classes of day-care centers, (ii) the ratio of day-care providers to children was identical, regardless of the size of the day-care center and (iii) the number of toys was similar in day-care centers, the lack of glove use could explain the more frequent transmission of CMV through urine in children attending large day-care centers.

During the 4-month period of surveillance, nine outbreaks of respiratory viral infections were recorded in eight day-care centers. The number of ARI outbreaks was six times higher in large day-care centers (6 episodes arose in December 1992 in day-care centers enrolling over 40 children vs. 1 episode in each of the other day-care centers enrolling 10–20, 21–30 and 31–40 children, respectively). All recorded viral etiologies have, for a long time, been known to cause ARIs, including echovirus type 11, which has been previously demonstrated to cause pharyngitis in children over 6 months of age [26]. This is in agreement with our results, which showed that echovirus type 11-related rhinopharyngitis was observed in children over 8 months of age. Because outbreaks due to respiratory viruses were significantly more frequently found in day-care centers in which CMV and respiratory viruses cocirculated, and, since viral ARIs were significantly more frequently reported in CMV-infected children, we suggest that viral ARIs are significantly more likely to occur in CMV-infected children.

The percentage (87.6%) of RSV-related bronchiolitis cases observed in children who presented with both CMV and RSV infections was significantly ($P < 0.001$) higher than the percentage of 15% observed in a previous study implemented in Lyon in children under 1 year of age for whom a physician was consulted [27]. Two hypotheses could explain the association between an incidental CMV infection and the development of viral ARI. First, CMV may induce transient immunosuppression and increase both the frequency and/or the severity of viral ARI, as has previously been observed [15, 16]. Secondly, respiratory viruses may lead to the reactivation of latent CMV. Among the 15 children with RSV infection, only one child became a CMV excretor. Since we have demonstrated that RSV-related bronchiolitis cases were significantly more frequent in children who became CMV positive, we favor the first hypothesis. Thus, CMV could act as an immunomod-

ulator and promote both the transmission and pathogenicity of RSV among young children. The number of children who presented with viral ARI of another etiology was too small to permit a statistical analysis.

We conclude that a significant percentage of children under the age of 1 year attending a large day-care center are likely to acquire CMV infection after a short period of time and that incidental CMV infection appears to favor the development of viral ARI, mainly that due to RSV.

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