Congenital cytomegalovirus infection in newborn infants of mothers infected before pregnancy*

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SUMMARY The rate of congenital cytomegalovirus (CMV) infection was studied in newborn infants in an African population in which all adults had experienced primary CMV infection during childhood. Viruria within the first 12 hours after delivery was taken as evidence of prenatal CMV infection. 28 of 2032 newborn infants examined had viruria, giving a rate of 1.4% congenital CMV infection. The presence of maternal serum antibody therefore appears not to protect the fetus from intrauterine infection. Either reactivation of latent maternal CMV infection or recurrence of infection during pregnancy despite the presence of serum antibodies may explain these findings. Whether the long-term effects of CMV infection acquired *in utero* differ in cases of primary maternal infection from those due to reactivated or recurrent infection in seropositive mothers, remains undecided. Thus, the value of a live CMV vaccine to prevent prenatal CMV infection may be questioned.

Cytomegalovirus (CMV) infection is the most common congenital infection (Klein *et al.*, 1976). It has been assumed that the virus gains access to the fetus mainly during primary infection of the pregnant woman (Stern and Tucker, 1973). However, there are also reports of prenatal CMV infection in newborn infants of mothers who had experienced primary CMV infection before pregnancy (Embil *et al.*, 1970; Krech *et al.*, 1971b; Stagno *et al.*, 1973). Thus the role of maternal immunity in protecting the fetus from being infected *in utero* is not understood. Nor is the significance of prenatal CMV infection in newborn infants of mothers who are seropositive before pregnancy yet established. Therefore, the study of the rate of congenital CMV infection in a population in which all adults show evidence of past CMV infection may lead to a better understanding of host immunity to CMV. We have already surveyed an African population to determine the prevalence of CMV infection as measured by the presence of serum antibodies (Krech, 1973). Congenital infection was assessed by the isolation of CMV from infants within the first hours of delivery.

Materials and methods

Five hundred male blood donors attending the Centre de Transfusion in Abidjan, Ivory Coast, and 338 apparently healthy children aged between 4 and 16 years were serologically screened for complement-fixing (CF) CMV antibodies. Blood specimens from these children were obtained during routine laboratory examinations for parasitic diseases. 2155 urine samples from babies were collected into urine bags within the first 12 hours of delivery. From the babies screened for viruria 1038 cord blood samples were available for serological testing. Head circumference and weight of all babies were recorded, and the age and the number of pregnancies of their mothers. The mothers had been admitted to the hospital just before delivery and most of them left the same day with the infant; unfortunately, no other controls could be included.

Serological studies. All sera were stored at -20°C until tested for CF antibodies against CMV antigen. Twofold dilutions of heat-inactivated serum were assayed in microtitre plates in accordance with a method using a glycine-extracted tissue culture antigen (Krech *et al.*, 1971a). An antibody titre of 1:4 or greater was considered as evidence of past CMV infection.

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*This study represents phase three within the CMV investigation programme of WHO (Krech, 1973)

Virological studies. Urine samples were mixed with an equal volume of a transport medium (35% sorbitol in Eagle's minimal essential medium enriched with 2% fetal calf serum, amino-acids, and vitamins) penicillin, streptomycin, gentamicin, kanamycin, and mycostatin were added. These samples were stored for up to 7 days at 4°C until shipped by air from Abidjan to Switzerland direct without refrigeration; they arrived at the institute within 3 or 4 days. Human fibroblast monolayers were inoculated with aliquots of urine (Krech *et al.*, 1971a). The cultures were kept for at least 7 weeks. For further identification the infected cells were observed by indirect immunofluorescence staining using a human serum with a high CF CMV antibody titre (1:240) and containing no antibodies against herpes viruses or adenoviruses when examined by immunofluorescence.

Results

Serological studies. 1876 sera were available. Of these 1395 (74.4%) could be used for assaying CF CMV antibodies. 481 samples were anticomplementary. The prevalence of CMV antibodies in the different population groups is shown in the Table. At 14 years, the age of the youngest pregnant woman in this study, 99.7% of the subjects tested were seropositive for CF CMV antibodies, so that virtually all women had experienced primary CMV infection before pregnancy. In only one cord blood out of 702 were CMV antibodies absent. There was an unequal distribution of CMV antibody titres in the serum samples of the different population groups tested (Fig. 1). The highest titres were found in cord blood, the lowest in the children aged between 4 and 8 years.

Virological studies. Among 2155 urine samples collected 123 (5.7%) were either contaminated or otherwise toxic to human fibroblasts. CMV was isolated from 28 samples giving a rate of 1.4% prenatal CMV infection. Cytopathogenic changes

Fig. 1 CF CMV antibody titres in different population groups. The highest titres are found in cord blood. The youngest children show the lowest titres (although the incidence of primary infection in this age group is high).

appeared as early as 10 days or as late as 48 days after inoculation.

Clinical findings. No apparent malformation, overtly sick child, or death was recorded among the babies examined. There were no differences in the head circumferences (CMV+ 33.81 ± 1.54 cm, $n=28$; CMV- 33.71 ± 1.21 cm, $n=200$; mean $\pm$ 1SD) or weights (CMV+ 2995 ± 455 g; CMV- 3050 ± 418 g) between the infected (CMV+) and 200 randomly selected noninfected (CMV-) infants. Head circumferences >1 SD above the mean (36, 36, 36, and 37 cm) were noted in 4 (14%) CMV+ babies, whereas in only 6 (3%) CMV- babies the same findings were observed (5 with 36 cm, one with 37 cm head circumference). No microcephaly was noted.

There was an equal distribution of mothers delivering CMV excretors compared with mothers with babies without viruria as far as age and number of pregnancies was concerned.

Discussion

In this survey of the rate of congenital CMV infection in an African population with a high incidence of CMV infection, measured by the presence of serum CF antibodies, a rate of 1.4% prenatal CMV infection was found. Urinary CMV excretion within the first 12 hours of delivery was taken as evidence for congenital CMV infection, and the rate of 1.4% probably somewhat underestimates the true incidence, since failure to isolate the virus owing to nonoptimal conditions of sampling and delay in posting is likely.

A comparison with the findings in Switzerland is illustrated in Fig. 2 (Krech and Jung, 1971; Krech *et al.*, 1971a). It can be seen that there are almost no

Table CF CMV antibodies in different population groups

	n*	Seropositive† No. (%)
Children	292	269 (92)
4-8 years	158	143 (91)
9-16 years	134	126 (94)
Blood donors	495	495 (100)
Cord blood	702	701 (100)

*Number of serum samples evaluated. †Titre of 1:4 or greater considered evidence of past CMV infection.

Fig. 2 CF CMV antibody prevalence and the role of prenatal CMV infection in Ivory Coast and Switzerland. Prenatal CMV infections are more common in a population (Ivory Coast) where all adults show evidence of past CMV infection.

primary CMV infections in the adult African population as measured by the presence of CF CMV antibodies. Yet, despite the lack of primary CMV infections in adults, the rate of congenital CMV infection is much higher in African than in Swiss babies. These data together with the observations of congenital CMV infections in siblings of consecutive pregnancies (Embil *et al.*, 1970; Krech *et al.*, 1971b; Stagno *et al.*, 1973) and the recent results of Stagno *et al.* (1977) seem to indicate that the presence of maternal CF antibodies to CMV does not protect the fetus from becoming infected. Stagno *et al.* (1977) found that in more than 3.4% of babies excreting CMV within the first week of delivery, in a selected population, the mothers had been seropositive before pregnancy. Congenital CMV infection does not necessarily produce congenital CMV disease; CMV infection of the fetus in mothers whose primary CMV infection occurred before conception may not be clinically significant (Krech *et al.*, 1971b; Stagno *et al.*, 1973), but long-term follow-up will be needed to determine this question. While many CMV-infected babies are apparently healthy at birth (Starr *et al.*, 1970), late sequelae affecting mainly mental development are observed (Hanshaw *et al.*, 1976) and may be related to subclinical encephalitis after CMV infection *in utero*. We also cannot rule out the possibility that the slightly increased head circumference in 4 (14%) of 28 congenitally infected babies in this study may be related to CMV disease.

Infection of the fetus in mothers who gave seropositive results could be explained either by reactivation of latent maternal CMV infection during pregnancy, or by reinfection in spite of the presence of serum antibodies. Different strains of CMV might also be responsible for recurrence of infections (Huang *et al.*, 1976). There are, however, only

minor antigenic differences between different strains of CMV and cross-immunity occurs (Andersen, 1970; Weller, 1971). Furthermore, genetic and antigenic homology has been demonstrated in CMV strains isolated from prenatally infected babies and their mothers (Stagno *et al.*, 1973, 1977). If primary CMV infection of the mother before pregnancy does not protect the infant from being infected *in utero* by CMV, then the value of a live CMV vaccine in preventing congenital CMV disease must be questioned (Elek and Stern, 1974). If, on the other hand, the presence of serum antibodies could protect against primary CMV infection, then immunisation with noninfective subunits of CMV, conferring immunity without producing infection, may be more promising (Pagano, 1976).

In this survey higher CF CMV antibody titres were observed in women at the end of pregnancy compared with the titres in male blood donors and children (Fig. 1). This observation, at present unexplained, might be related to alterations of virus-host interaction during pregnancy. The role of cell-mediated immunity in CMV infections also requires further investigation. There is evidence of some impairment of cell-mediated immune mechanisms in mothers delivering prenatally infected babies (Rola-Pleszczynski *et al.*, 1977).

In conclusion, host immunity to CMV infection may be more complex than was previously appreciated. A better understanding of the immune mechanisms may open the way to effective prevention of prenatal CMV infection with its toll of mental retardation (Weller, 1971; Elek and Stern, 1974).

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