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Intra-uterine transmission of cytomegalovirus in women known to be immune before conception

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SUMMARY

A prospective study identified 785 pregnant women who had been shown to possess complement fixing antibodies against cytomegalovirus (CMV) during a previous pregnancy. As these women were thus known to have been immune prior to their subsequent conception, their neonates were examined for evidence of congenital CMV infection. Specimens were obtained from 725 (92%) of the neonates and congenital infection was found in only one (0·14%). The elder sister of the infected child was also shown, by retrospective testing of her stored cord serum for specific IgM antibodies, to have been infected *in utero*. Thus, one woman was identified who had delivered consecutive siblings congenitally infected with CMV. We conclude that some women have a propensity for intra-uterine transmission of CMV, despite being immune prior to conception, and speculate that such women may have acquired their infections perinatally.

INTRODUCTION

Cytomegalovirus (CMV) is a member of the herpesvirus family and, like all herpesviruses, it persists in the host after primary infection. The virus can reactivate from the latent state at unpredictable intervals and nearly always remains asymptomatic. Reactivations occurring in pregnant women were not thought to represent a threat to the fetus since it should be protected by the mother's pre-existing antibodies (Stern & Tucker, 1973). However, three case reports from Canada (Embil, Ozere & Haldane, 1970), Switzerland (Krech, Konjajev & Jung, 1971) and Alabama (Stagno *et al.* 1973) have demonstrated that congenital infection can occur in consecutive siblings, presumably due to reactivation of latent maternal infection (Huang *et al.* 1980). It was generally assumed that this must be a rare event but the Alabama workers have subsequently provided evidence that, in their highly seropositive population, maternal recurrences accounted for more cases of congenital infection than did primary maternal infections (Stagno *et al.* 1977). However, the population studied in Alabama is one of particularly low socio-economic background with a relatively high rate of congenital CMV infection and it would be unwise to assume that their results could

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be applied to the obstetric patients seen in England. We therefore decided to perform a study of congenital infection in babies born in this country whose mothers were known to possess CMV antibodies before they conceived.

MATERIALS AND METHODS

Population studied

Between 1 February 1977 and 31 July 1982 women were identified when they returned to this hospital for further antenatal care having had a previous pregnancy monitored for evidence of primary CMV infection as part of another study (Griffiths & Baboonian, 1983). A total of 1570 women with sequential pregnancies were identified of whom 1440 (91·8%) delivered at this hospital between 10 August 1977 and 16 December 1982. Of these women, 785 (54%) had been shown to possess CMV complement fixing antibodies during their previous pregnancy and so were entered into the study reported here. Congenital infection was sought in their neonates by attempting to culture CMV from urine samples and/or by demonstrating the presence of CMV-specific IgM antibodies in cord sera.

Culture of virus

Specimens of fresh urine from neonates less than one week of age were collected by us from the hospital wards and were inoculated immediately into 5–6 replicate cell culture tubes of low-passage human embryo lung fibroblasts. Thrice weekly for 21 days the cultures were examined for the cytopathic effect typical of CMV.

Serological methods

Complement fixing (CF) antibodies against CMV were measured in sera diluted 1 in 8 as described previously (Griffiths, Buie & Heath, 1978).

Antibodies of IgM class were measured by solid-phase radioimmunoassay using the conditions described for adult sera (Kangro, 1980) or for cord sera (Griffiths *et al.* 1982) as appropriate. To exclude rheumatoid factor activity, all samples reacting in the radioimmunoassay were confirmed by fractionation through Sephadex G-200 (Pharmacia) columns together with retesting of the serum IgM fraction by radioimmunoassay as previously described (Griffiths, 1981).

HLA typing

HLA antigens were detected on peripheral blood leucocytes by a lymphocytotoxicity assay (Festenstein *et al.* 1972).

RESULTS

Patients investigated

During this study, clinical samples were obtained from 725 (92%) of the 785 neonates born to seropositive women. Both urine and cord blood were obtained from 445 neonates while urine or cord serum only were obtained from 108 and 172 neonates respectively.

Cytomegalovirus was cultured from only one of the 553 urine samples. Specific IgM antibodies were found in only one of the 617 cord sera tested; this sample had

Table 1. *Results obtained by testing cord sera and serial maternal sera taken during both pregnancies*

Date	Gestational stage (weeks)	Antibody titres CF	Antibody titres RIA-IgM
First pregnancy			
16. 6. 78	8	32	< 100
14. 7. 78	12	32	< 100
5. 12. 78	33	128	< 100
16. 1. 79	38 (cord)	n.d.	4000
18. 1. 79	38 (maternal)	256	< 100
Second pregnancy			
12. 10. 79	20	128	< 100
7. 1. 80	32	128	< 100
29. 2. 80	40 (cord)	n.d.	800
1. 3. 80	40 (maternal)	64	< 100

n.d. = not done.

been collected from the baby shown to be excreting virus at birth. Thus, only one case of congenital infection was documented in the 725 neonates examined (0·14 %). The identification of this case prompted a full review of the maternal history and laboratory findings which is presented below.

Case report

A 27-year-old Caucasian schoolteacher, gravida 2 para 0, booked for antenatal care on 16 June 1978 with amenorrhoea of 8 weeks' duration. The pregnancy was uneventful and she went into spontaneous labour at 38 weeks. Simpson's forceps were applied because of delay in the second stage of labour together with fetal distress and a live baby girl was delivered. The birth weight was 2·78 kg and the neonatal period was complicated only by jaundice between days 3 and 5 (peak serum bilirubin 176 μmol/l on day 3).

This woman re-booked for antenatal care on 12 October 1979 at 20 weeks. The pregnancy was uneventful and spontaneous delivery of a baby girl occurred at term. The birth weight was 3·52 kg and 'mild' jaundice was noted between days 3 and 5; serum bilirubin was not measured. As described above, a urine sample had been obtained from this baby 5 days after delivery and CMV was cultured from it.

Sera taken during both pregnancies were tested for the presence of CMV antibodies. The results in Table 1 show that there was serological evidence of CMV reactivation in the mother's first pregnancy whilst her CF antibodies declined slightly during the second pregnancy. Specific IgM antibodies were not found in any of the maternal sera but were present in *both* cord sera. Thus, this woman had delivered consecutive siblings congenitally infected with CMV.

Both the elder and younger siblings are being followed as part of our long-term paediatric investigation into the effects of CMV infection during pregnancy. When last seen at the ages of 4 years 3 months and 3 years 2 months respectively both children were developing normally. When tested at the ages of 3 years and 3 years 2 months respectively the two children achieved above-average scores on the

Table 2. *Histocompatibility antigens detected in the index family*

Subject	Genotype		
Father	A1	B8	(BW6) CW7
	A2	B27	(BW4) CW3
Mother	A3	B7	(BW6) C7
	A2	BW57	(BW4) C6
Elder sibling	A2	B27	(BW4) CW3
	A2	BW57	(BW4) C6
Younger sibling	A1	B8	(BW6) CW7
	A3	B7	(BW6) C7

Reynell Developmental Language Scale (standard scores +0·9 and +1·9 for verbal comprehension and +0·1 and +2·1 for expressive language respectively).

Genetic studies in index family

To determine if this family possessed an HLA-type reported to be associated with CMV infection, and if inheritance of a particular haplotype segregated with virus transmission *in utero*, the whole index family was typed at the HLA-A, B and C loci. The results in Table 2 show that the two siblings do not share A, B or C HLA antigens. Neither sibling inherited her histocompatibility antigens by way of a cross-over.

DISCUSSION

This study, involving the recruitment and virological investigation of over 700 neonates, has conclusively demonstrated that, in the population of women being studied in London, the possession of specific humoral immunity prior to conception cannot prevent CMV from being transmitted *in utero*. The diagnosis of congenital infection in the index case is unequivocal since CMV was cultured from a urine sample taken within one week of birth and specific IgM antibodies were detectable in cord serum by a radioimmunoassay which has been fully investigated in a virologically defined population of neonates, including those infected as a result of recurrent maternal infection (Griffiths *et al.* 1982).

The finding of this case should not have been unexpected since similar results have been reported from other countries (Embil, Ozere & Haldane, 1970; Krech, Konjajev & Jung, 1971; Stagno *et al.* 1973). Nevertheless, some British authors have denied the possibility that recurrent maternal infections might result in intra-uterine transmission in this country. In 1973, Stern & Tucker examined the infants of eight women who had had viruria during pregnancy and were able to show that virus transmission *in utero* had not occurred. More recently, Grant, Edmond & Syme (1981) identified a group of 12 women with viruria whose booking sera contained complement fixing antibodies but not IgM antibodies detectable by immunofluorescence. They considered that these women had reactivated CMV infections and found no evidence of congenital infection in their neonates. Both of these studies can be criticized for their small number of patients as well as their definition of recurrent maternal infection, since the women identified may have had primary infections early in pregnancy. Furthermore, it is well recognized that

excretion of CMV from the cervix or urine is a poor indicator of which women will ultimately deliver congenitally infected babies (Reynolds *et al.* 1973).

Having established that some cases of congenital CMV infection in this country result from recurrent maternal infection, it will be important to define how frequently this occurs. At present, there is no laboratory test which can be applied to immune women to determine in which ones CMV transmission is occurring *in utero*; the second pregnancy in the woman reported here illustrates that rising titres of CF antibody cannot be used for this purpose. However, it is now possible to diagnose recurrent maternal infections with intra-uterine transmission by exclusion of primary infection. Provided that they first attend within 16 weeks' amenorrhoea, the absence of specific IgM antibodies in the booking sera of mothers of congenitally infected babies can provide a presumptive diagnosis of recurrent CMV infection during pregnancy (Griffiths, 1983). This interpretation of the serological response to CMV infection is illustrated by the mother reported here who did not produce detectable levels of specific IgM antibodies during two pregnancies where intra-uterine transmission of CMV occurred. Her second pregnancy is a case of proven recurrent maternal infection while her first pregnancy is classified as presumptive recurrent (Griffiths, 1983). This diagnostic approach has been used recently by another group of investigators in West London to provide preliminary evidence that recurrent maternal infection may be responsible for approximately one quarter of cases of congenital infection seen in this country (Peckham *et al.* 1983).

As regards the clinical effects of this infection the two cases reported here have achieved their expected clinical milestones and, unlike those children in the same population infected by way of primary maternal infection (Preece *et al.* 1983) do not have evidence of more subtle impairments of their speech and language development. However, many more cases like these must be identified and followed-up before it can be claimed that congenital infection resulting from maternal reactivation is less severe than that resulting from primary or undefined maternal infection (Hanshaw *et al.* 1976; Alford, Stagno & Pass, 1980; Saigal *et al.* 1982; Peckham *et al.* 1983; Preece *et al.* 1983).

Although we expected this study to identify a case of recurrent maternal infection with transmission, it was surprising to find that an earlier sibling had also been congenitally infected. This represents the first report from this country of congenital CMV infection in sequential siblings, and strongly suggests that some women have a predisposition towards this type of transmission *in utero*. Such a predisposition could be genetic, and two groups of workers have suggested that particular HLA antigens may be associated with CMV infection (Pereira, James & Stern, 1978; Stagno *et al.* 1980). Neither of the two cases reported here had the HLA-types identified by these workers.

While the explanation for intra-uterine transmission of virus in seropositive women thus still remains elusive, it is interesting to compare the results from our population with those of two populations of women studied concurrently in urban Alabama (Stagno *et al.* 1982). Immune women from a population with a low socio-economic background and a CMV seropositivity rate of 82%, transmitted virus *in utero* in 1·5% (13/835) of cases; a rate which is significantly ($P < 0{\cdot}005$) different from that reported here (0·14%; 1/725). In contrast, immune women from a middle-income population with a seropositivity rate of 55% transmitted virus

less frequently (0·5%; 7/1495), a rate which was not significantly different from our results. These data show that differences between the two countries cannot be explained on a geographical basis and lead us to suggest a possible explanation. Since disadvantaged societies with a high CMV seropositivity rate have a high incidence of perinatal CMV infection (Reynolds *et al.* 1973; Alford, Stagno & Pass, 1980) we speculate, as has Stern (1977) before us, that children infected at this early age may be those who, many years later, transmit the virus *in utero* despite having humoral immunity. Acquisition of CMV in the presence of passively acquired antibody at a time when the neonatal cell-mediated immune response is immature may lead to an unstable host–parasite relationship with a propensity for virus reactivation later in life. Thus, by analogy with hepatitis B virus, perinatal CMV infection may be benign in its acute phase, but we speculate that it may produce adverse long-term sequelae in terms of subsequent transmission *in utero* when the infants reach reproductive age.

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REFERENCES

ALFORD, C. A., STAGNO, S. & PASS, R. F. (1980). Natural history of perinatal cytomegaloviral infection. In *Perinatal Infections*, pp. 125–147. Ciba Foundation – Excerpta Medica, Amsterdam.

EMBIL, J. A., OZERE, R. L. & HALDANE, E. V. (1970). Congenital cytomegalovirus infection in two siblings from consecutive pregnancies. *Journal of Pediatrics* **77**, 417–421.

FESTENSTEIN, H., ADAMS, E., BURKE, J., OLIVER, R. T. D., SACKS, J. A. & WOLFE, E. (1972). The distribution of HL-A antigens in ex-patriots from East Bengal living in London. In *Histocompatibility Testing* (ed. J. Dausset and J. Colombani), pp. 175–178. Copenhagen: Munksgaard.

GRANT, S., EDMOND, E. & SYME, J. (1981). A prospective study of cytomegalovirus infection in pregnancy. *Journal of Infection* **3**, 24–31.

GRIFFITHS, P. D. (1981). The presumptive diagnosis of primary cytomegalovirus infection in early pregnancy by means of a radioimmunoassay for specific IgM antibodies. *British Journal of Obstetrics and Gynaecology* **88**, 582–587.

GRIFFITHS, P. D. (1983). Congenital cytomegalovirus infection. In *Recent advances in Clinical Virology*, vol. 3 (ed. A. P. Waterson), pp. 57–71. Edinburgh: Churchill Livingstone.

GRIFFITHS, P. D., BUIE, K. J. & HEATH, R. B. (1978). A comparison of complement fixation, indirect immunofluorescence for viral late antigens and anti-complement immunofluorescence tests for the detection of cytomegalovirus specific serum antibodies. *Journal of Clinical Pathology* **31**, 827–831.

GRIFFITHS, P. D. & BABOONIAN, C. (1983). A prospective study of primary cytomegalovirus infection during pregnancy: final report. *British Journal of Obstetrics and Gynaecology* (in the Press).

GRIFFITHS, P. D., STAGNO, S., PASS, R. F., SMITH, R. J. & ALFORD, C. A. (1982). Congenital cytomegalovirus infection: diagnostic and prognostic significance of the detection of specific IgM antibodies in cord serum. *Pediatrics* **69**, 544–549.

HANSHAW, J. B., SCHEINER, A. P., MOXLEY, A. W., GAEV, L., ABEL, V. & SCHEINER, B. (1976). CNS effects of 'silent' cytomegalovirus infection. *New England Journal of Medicine* **295**, 468–470.

HUANG, E.-S., ALFORD, C. A., REYNOLDS, D. W., STAGNO, S., PASS, R. F. (1980). Molecular epidemiology of cytomegalovirus infections in women and their infants. *New England Journal of Medicine* **303**, 958–962.

KANGRO, H. O. (1980). Evaluation of a radioimmunoassay for IgM class antibodies against cytomegalovirus. *British Journal of Experimental Pathology* **61**, 512–520.

KRECH, U., KONJAJEV, Z. & JUNG, M. (1971). Congenital cytomegalovirus infection in siblings from consecutive pregnancies. *Helvetica Paediatrica Acta* **26**, 355–362.

PECKHAM, C. S., CHIN, K. S., COLEMAN, J. C., HENDERSON, K., HURLEY, R. & PREECE, P. M. (1983). Cytomegalovirus infection in pregnancy: preliminary findings from a prospective study. *Lancet* i, 1352–1355.

PEREIRA, R. S., JAMES, D. C. O. & STERN, H. (1978). Correlation between cytomegalovirus infection and HLA-BW15. *British Medical Journal* ii, 126.

PREECE, P. M., BLOUNT, J. M., GLOVER, J., FLETCHER, G. M., PECKHAM, C. S. & GRIFFITHS, P. D. (1983). A study of children exposed to primary maternal cytomegalovirus infection during pregnancy. *Archives of Disease in Childhood* (in the Press).

REYNOLDS, D. W., STAGNO, S., HOSTY, T. S., TILLER, M. & ALFORD, C. A. (1973). Maternal cytomegalovirus excretion and perinatal infection. *New England Journal of Medicine* **289**, 1–5.

SAIGAL, S., LUNYK, O., LARKE, R. P. B. & CHERNESKY, M. A. (1982). The outcome in children with congenital cytomegalovirus infection. *American Journal of Diseases in Children* **136**, 896–901.

STAGNO, S., REYNOLDS, D. W., LAKEMAN, A., CHARAMELLA, L. J., ALFORD, C. A. (1973). Congenital cytomegalovirus infection; consecutive occurrence due to viruses with similar antigenic compositions. *Pediatrics* **52**, 788–94.

STAGNO, S., REYNOLDS, D. W., HUANG, E.-S., THAMES, S. D., SMITH, R. J. & ALFORD, C. A. (1977). Congenital cytomegalovirus infection: occurrence in an immune population. *New England Journal of Medicine* **296**, 1254–1258.

STAGNO, S., SHAW, J. F., PASS, R. F. & ALFORD, C. A. (1980). HLA types in symptomatic congenital cytomegalovirus infections. *Pediatric Research* **14**, 565.

STAGNO, S., PASS, R. F., DWORSKY, M. E., HENDERSON, R. E., MOORE, E. G., WALTON, P. D. & ALFORD, C. A. (1982). Congenital cytomegalovirus infection: the relative importance of primary and recurrent maternal infection. *New England Journal of Medicine* **306**, 945–949.

STERN, H. (1977). Cytomegalovirus infection in the neonate and its prevention. *Postgraduate Medical Journal* **53**, 588–591.

STERN, H. & TUCKER, S. M. (1973). Prospective study of cytomegalovirus in pregnancy. *British Medical Journal* ii, 268–270.