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Identification of pregnancies at risk from cytomegalovirus infection

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SUMMARY

The fluorescent antibody technique was used for the identification of specific cytomegalovirus IgM in the sera of twenty-four of 1065 unmarried pregnant women. Seventeen of them were followed to term and five infected infants were identified. Two other infants had CMV IgM in neonatal serum samples but virus excretion was not demonstrated. The congenital infection rate in this study was 5·3 per 1000 births by virus excretion and 7·9 per 1000 if cases with specific IgM are included; from previous studies a rate of 8·8 per 1000 was expected. The reasons for the lack of relationship between specific IgM in the mothers' serum and infected babies is discussed.

INTRODUCTION

Unlike rubella infection cytomegalovirus (CMV) usually gives no recognizable or suggestive clinical syndrome which would indicate that infection had occurred, so pregnancies at risk from this virus are unlikely to be found or suspected clinically.

After CMV infection, an antibody response takes place in all immunoglobulin fractions. IgG antibody probably persists for life, IgA for a year or more and IgM for up to eight months (Schmitz & Haas, 1972). Reactivation or reinfection by CMV can also give a serological response, but whether the IgM fraction is involved or not has not been definitely determined except for immunosuppressed patients (Nagington, 1971).

If specific IgM antibody against CMV could be demonstrated in ante-natal sera it would suggest that the patient had been infected within eight months of the date of blood testing and that her fetus could have been infected in utero (Tobin, 1973). A search for such pregnancies has been made, mainly amongst unmarried pregnant women who have an increased risk of producing congenitally infected infants as compared with married ones (Collaborative Study, 1970).

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METHODS

Ante-natal sera from 1065 unmarried pregnant women and from 690 married pregnant women were obtained from the ante-natal clinics of St Mary's, Withington and Wythenshawe Hospitals, through the courtesy of Dr P. J. L. Sequeira, Director of the Central Serology Laboratory. These sera were screened at a dilution of 1/4 or 1/5 for the presence of either complement fixing (C. F.) or fluorescent IgG antibody to CMV. From those with no detectable antibody a second serum was requested at a later ante-natal visit around the 32nd week of pregnancy, and the two specimens were tested in parallel by the C.F. test in order to detect any rise in titre. Serum from those unmarried mothers who already had CMV antibody in the screening test was further tested for the presence of specific CMV IgM antibody by immunofluorescence.

CMV-infected monolayers on teflon-coated slides, fixed in acetone and kept at −20 °C until required, were used for the immunofluorescent tests. (de Silva, Khan, Kampfner & Tobin, 1973.) Serum dilutions in 0·02 ml amounts were left in contact with monolayers for 30 min at 37 °C for IgG estimations and either for 3 h at 37 °C or overnight at 4 °C for IgM estimations. Overnight contact was used for infant sera. After two 5 min washings with phosphate buffered saline (PBS) the slides were dried and 0·02 ml of the relevant antiglobulin conjugate added (Wellcome Reagents or Hyland Laboratory products) and allowed to react for sixty minutes at 37 °C. After washings with PBS as before, slides were mounted in 50% glycerol in carbonate-bicarbonate buffer pH 9·5 and examined microscopically.

Any degree of fluorescence, however doubtful, suggesting a positive reaction initiated a request for a second serum. On receipt of the second serum the two were again screened for specific IgM at a dilution of 1/3 or 1/5 and those found to be positive were studied further by titrating at 3-fold dilutions the concentration of IgM and IgA in neat serum and in fractions obtained by sucrose density gradient centrifugation (SDGC).

Sucrose gradients were made in 5·5 ml centrifuge tubes, using five concentrations of sucrose in PBS (37·5 to 12·5%) and floating 0·1–0·25 ml of serum in a total of 0·5 ml of serum/PBS mixture on top. Centrifugation was done at 40,000 rev./min for seventeen hours in a swing-out head using an MSE Superspeed 65 (135,000 **g**). Twelve fractions were collected, the IgM being mostly in fraction 3, and IgG in fraction 5 onwards. Specific CMV antibody was detected in the fractions by immunofluorescence as described above.

Serum giving a positive reaction for IgM was also tested for anti-IgG activity by the Standard Latex test (Wellcome Reagents Ltd) and for sheep cell agglutination by the 'monospot' technique. Urine and throat swabs from these women were requested at their second ante-natal visit, as were throat swabs and urine for virus isolation and blood for serological studies from them and their babies after delivery.

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Table 1. *Summary of laboratory results*

(1) No. tested	1065
(2) No. with CMV antibody	682
(3) No. with ? possible CMV IgM	108
(4) No. with confirmed CMV IgM	24
(5) No. of (4) followed to term	17
(6) No. of (5) with IgM in SDGC	7 (3)*
(7) No. of (5) with IgM not in SDGC	9 (1)*
(8) No. of (5) not tested by SDGC	1

* Number of pregnancies with babies excreting CMV.

RESULTS

A total of 1065 unmarried pregnant women were screened for CMV antibody by either complement fixation or immunofluorescence and requests for a second serum in 164 of those without C.F. antibody were made. A second serum was received from seventy of them. Only one (case I in Table 2) showed a four-fold or more rise in complement-fixing antibody titre, together with the presence of specific antibody in both the IgA and IgM serum fractions. This patient produced an infected infant. Similar requests for a second serum from 296 married pregnant women without C.F. antibody were made and 167 were received. None of these women developed antibody between the taking of the two serum samples.

Of the 1065 unmarried women tested 682 had complement-fixing or fluorescent IgG antibody in their serum, and 108 showed some reaction in the IgM immunofluorescence (IF) test (Table 1). A second serum was requested from them, however doubtful the fluorescence was considered in the initial sample, and 81 second sera were received. Samples from 79 women were considered to be 'false' positives as repeated tests on first, and second sera when received, failed to show any specific immunofluorescence with the IgM conjugate and/or had no specific IgA IF antibody. Forty-nine were followed to term, as were five whose IgM fluorescence was due to rheumatoid factor, and none of the babies were found to be excreting CMV. The virus was found in the urine and/or throat swab of five of fifty-five of those mothers tested at some time during pregnancy. No sera reacted in the monospot test.

Twenty-four women had specific fluorescent IgA and IgM in their ante-natal serum; seventeen of these were followed to term and their babies examined for virus excretion, and in some cases for specific IgM and IgA, in their blood. Seven of these seventeen (Table 2, Cases 1, 2, 4, 5, 7, 8, 11) had specific CMV IgM confirmed in the relevant SDGC fractions and three of these (Cases 1, 2 and 4) produced four infected infants, one woman having twins. Two of this group of mothers yielded CMV at some time during their pregnancy from urine or throat. The nine of the other women tested did not have specific CMV IgM confirmed in their serum by SDGC, and one of these produced an infected infant (Case 3). The first blood of this woman was positive in the screening IF test, but it could not be tested by SDGC: and both subsequent samples were found negative by SDGC although the earlier one of these two was weakly positive by immunofluorescence of the whole serum.

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Table 2. *Seventeen CMV-specific IgM positive cases followed to term*

		Mother							Baby					
			Serology						Serology					
				IF.						IF.				
						SDGC						SDGC		
Case no.	Age	Weeks gestation & after delivery (+)	C.F.	Whole IgA	Serum IgM	IgA	IgM	Virus isolation. T/S and/or urine	C.F.	Whole IgA	Serum IgM	IgA	IgM	Virus isolation. T/S and/or urine
1	19	29	< 5	45	≥ 45	NT	NT	NT						
		32	≥ 160	45	45	9	3	+						
		+ < 1	≥ 160	45	15	NT	NT	+	20	45	≥ 45	NT	NT	+
		+2	≥ 160	15	< 5	3	–							
2	26	25	≥ 160	NT	+	NT	NT	NT						
		+ < 1	≥ 160	15	45	NT	NT	–	≥ 160	45	+	45	–	+
		+1	≥ 160	≥ 45	45	+	18	–						
3	19	16	80	45	5	NT	NT	NT						
		25	80	45	≤ 5	+	–	+						
		+ < 1	80	45	–	+	–	–	30	NT	< 5	NT	NT	+
4	20	16	40	45	45	+	+	NT						
		28	40	45	15	9	+	–	(*a*) NT	45	5	NT	NT	+
									(*b*) NT	45	5	NT	NT	+
5	41	18	NT	≥ 45	5	NT	NT	NT						
		24	NT	≥ 45	5	9	3	–						
		+ < 1	NT	45	5	9	3	–	NT	+	+	NT	NT	–
6	24	17	20	15	5	3	–	NT						
		+ < 1	A/C	15	5	NT	NT	–	A/C	NT	+	NT	NT	–
7	23	10	10	NT	+	NT	NT	NT						
		29	40	45	45	9	3	–						
		33	40	45	45	9	3	–						
		+2	A/C	45	15	9	9	–	A/C	NT	–	NT	NT	–

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Table 2 (*cont.*)

8	26	20	≥ 160	15	15	NT	NT	NT						
		31	≥ 160	15	5	9	3	+						
		+1	≥ 160	45	5	9	+	–	NR					–
9	21	11	80	15	+	NT	NT	NT						
		21	160	45	15	9	–	–						
		+ < 1	80	45	5	NT	NT	–	NT	–	–	NT	NT	–
10	29	25	NT	15	15	NT	NT	NT						
		35	40	15	45	3	–	–						
		+ < 1	40	15	15	9	–	–	40	NT	–	NT	NT	–
11	21	25	40	45	15	NT	NT	NT						
		30	40	45	15	9	+	–						
		+ < 1	A/C	45	15	+	12	–	NR					–
12	24	14	40	15	5	NT	NT	NT						
		23	80	≥ 45	15	+	–	–						
		+ < 1	160	45	15	NT	NT	–	160	–	–	NT	NT	–
13	21	13	160	45	15	NT	NT	NT						
		+ < 1	160	45	45	+	–	–	NT	NT	–	NT	NT	–
14	27	13	A/C	≥ 45	+	NT	NT	NT						
		27	160	≥ 45	5	NT	NT	–						
		+ < 1	160	≥ 45	5	+	–	–	NT	NT	–	NT	NT	–
15	18	14	30	15	+	NT	NT	NT						
		23	60	45	5	NT	NT	–						
		+1	40	15	15	3	–	–	40	15	–	NT	NT	–
16	28	14	NT	NT	+	NT	NT	NT						
		27	NT	45	15	9	–	–						
		+ < 1	80	45	15	9	–	–	40	45	–	NT	NT	–
17	27	21	NT	+	+	NT	NT	NT						
		31	NT	15	45	NT	NT	NT						
		22	NT	15	45	NT	NT	NT	NR					–

Fluorescence detected (+) or not detected (–) at a dilution of 1:3. NR, not received; NT, not tested; A/C, anti-complementary; SDGC, serum fractionated by sucrose density gradient centrifugation.

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Four out of five babies (Cases 1, 2 and 4a and b) who were shown to be excreting cytomegalovirus also had specific IgM in their serum postnatally as did two babies (Cases 5 and 6) who were not found to be excreting virus; one mother (Case 5) had IgM antibody confirmed by SDGC but the other (Case 6) did not. Two babies (Cases 15 and 16) had CMV IgA, but not IgM in their serum.

DISCUSSION

These attempts to identify pregnancies at risk from congenital CMV infection have not been as clear-cut as might have been hoped. Of the seventeen women who, from laboratory tests, had results indicating recent infection and who were followed to term, only four produced infected infants. Stern & Tucker (1973) have shown that only half of the women infected in pregnancy produce infected infants, and as specific CMV IgM may last up to eight months it is possible that the remainder of the women were infected before conception. These two observations might explain partly why only four infected pregnancies were identified. As only seventeen out of twenty-four women (71 %) with CMV IgM ante-natally could be followed to term a calculation of the frequency of babies excreting virus neonatally should relate to 71 % of the total of 1065 examined. The rate of identification of pregnancies at risk therefore, as judged by virus excretion by the neonatal infant was 4/756 or 5·3 per 1000 in this study: this can be compared with the rate of 8·8 per 1000 unmarried mothers found in two studies in 1967 to 1969 (Collaborative Study, 1970) and from 1969 to 1973 (unpublished results). If the two babies with IgM in their neonatal serum, but who failed to yield virus (Hanshaw, Sternfield & White, 1968), are also considered as congenital infections this rate would be 7·9 per 1000.

Three women producing infected infants were apparently infected sometime in the first or second trimesters and the fourth woman had a rise in C.F. titre in the third trimester.

Schmitz *et al.* (1977) in Freiburg using the fluorescence technique found specific IgM in 7 % of the 629 patients studied by them, a rate higher than that found by us. They explain this high incidence by an increased rate of CMV reactivations during pregnancy. Five babies were found to have CMV IgM in their cord blood and subsequently to be excreting CMV, giving a congenital infection rate in their study of 7·9 per 1000 which is about the same as that found by us in Manchester.

For the screening of ante-natal serum for specific IgM antibody the immunofluorescent technique is the most practical one at the moment, but is not entirely satisfactory. It is complicated by 'false' positives which may be due to anti-Ig macroglobulins (Shirodaria, Frazer & Stanford, 1973) or anti-cell antibodies (Hanshaw, Niederman & Chessin, 1972) as well as to the degree of discrimination and overenthusiasm of the operator. Positive results should be confirmed by sucrose density gradient centrifugation and by the use of the Latex and monospot tests. Further experience with the I.F. test in conjunction with other techniques is obviously needed before widespread ante-natal testing for cytomegalovirus macroglobulins is introduced. The significance of CMV IgA in the newborn also needs further study.

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The identification of pregnancies at risk suggests the possibility of termination in these cases. If this is to be contemplated the sooner the infection in the mother is recognized the better, but in our series of unmarried mothers the majority reported to the ante-natal clinics in the second trimester. This makes termination technically and ethically difficult, although the use of prostaglandins has enabled this procedure to be performed safely up to the twentieth week of gestation (Embrey, 1975).

The identification before delivery of pregnancies at risk would enable the new infant to be followed up for signs of congenital defects and appropriate remedial treatment instituted.

Work on a cytomegalovirus vaccine is in progress (Elek & Stern, 1974*a*), but it will be some time before it has been adequately tested or is ready for use. The effectiveness of a vaccine may be difficult to assess. The women producing congenitally infected infants tend to be in the less well-informed groups in the community and it is possible that they will not readily volunteer for vaccination. In this study the infants excreting CMV were not followed up as they and their mothers disappeared after the immediate post-partum period and could not be traced by the local medical services. The question of risk from cancer in the use of a live herpesvirus vaccine has also to be considered, although this risk is not considered to be great (Elek & Stern, 1974*b*).

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