ANTIBODIES TO CYTOMEGALOVIRUS AND OTHER MEMBERS OF THE HERPES GROUP IN WOMEN AND CHILDREN IN SINGAPORE

By E. H. Sng and J. O'H. Tobin

SYNOPSIS

Antibodies to the cytomegalovirus appear to be acquired at an early age in a number of Asian and African populations. Using the complement-fixation and neutralisation tests, it was found that in Singapore 88% school-children and ninety-eight of ninety-nine expectant women had cytomegalovirus antibodies. The incidence of neutralisation antibody to herpes simplex virus and fluorescent antibody to Epstein-Barr virus in the children was 75% and 92% respectively, it is suggested that the close family contacts amongst Asians in Singapore might be partly responsible for the acquisition of the viruses by the population at an early age.

Cytomegalovirus (CMV) is a member of the herpes virus group, being similar in size and structure to herpes simplex virus (HSV). Classically its presence in tissues is characterised by the development of large cells with intranuclear inclusion bodies and sometimes cytoplasmic ones. The intranuclear inclusion body is separated from the nuclear membrane by a wide halo as seen under a light microscope and may stain with either basophilic or acidophilic dyes. Except in material from infants dying within a few weeks of birth, or those on immunosuppressive therapy, these inclusions are not always obvious. They are usually difficult to find in urine, or in biopsy or necropsy specimens although the patient may be excreting large amounts of virus.

A review of the congenital and acquired form of CMV infection has been given recently by Stern (1968). The congenital disease in its severe form is characterised by mental retardation associated with microsephaly, paraplegia and sometimes intracerebral calcification. Hepatosplenomegaly, jaundice, thrombocytopenia and other less specific symptoms may occur. Hare-lip, inguinal hernias and cardiac defects have been described as structural abnormalities due to CMV. However, it is becoming apparent that most congenital infections are either subclinical or not recognised and that the severer manifestations are the exception rather than the rule. Although many symptoms have been attributed to CMV the only acquired infection that is well-documentated is that of infectious mononucleosis associated sometimes with hepatitis or polyneuritis.

Antibodies to CMV are present in all populations so far studied, most of the infections apparently occurring without any recognisable symptoms. Once acquired, the antibodies are considered to persist for a long time if not for life.

This paper gives results of a serological survey of CMV antibodies in expectant mothers and school-children in Singapore. Results are compared with those in other places and the frequencies of antibodies to rubella in these women and to HSV and the Epstein-Barr virus (EBV) in these children are also noted.

MATERIALS AND METHODS

Sera were taken from 100 normal expectant mothers attending maternity clinics and from 50 normal school-children between the ages of 7 and 9 years. CMV antibodies were titrated by either the complement-fixation test (Report, 1970) or, if the sera were anticomplementary, by a neutralization test using the AD169 strain of CMV. Antibodies to HSV were also titrated by a similar neutralization test. In these neutralizing screening tests a single 1/6 dilution of serum was used; a 0·4 ml. volume of this dilution and 0·2 ml. of virus suspension containing 200 TCD_{50} of either CMV or HSV were added and the mixture allowed to react at 4°C overnight. 0·3 ml. of the serum dilution was added to two human fibroblast culture tubes per dilution and incubated at 36°C in roller drums. Neutralization was considered to have occurred if no virus CPE appeared within 7 days with HSV or in 10 days with CMV. This neutralization technique has been used successfully

Department of Pathology, General Hospital, Singapore.
E. H. SNG, M.B., B.S., Dp.Bact., Senior Registrar.

Public Health Laboratory, Manchester.
J. O'H. TOBIN, M.A., B.M., B.Ch.(Oxon), Dp.Bact.(Manch), M.R.C.Path., Director.

for titration of CMV and HSV antibodies in the Manchester Public Health Laboratory.

EBV antibody war detected by a fluorescent antibody (FA) technique similar to that described by Pereira *et al* (1969) using an initial dilution of 1/8.

RESULTS

Ninety-eight of 99 expectant mothers had detectable CMV complement-fixing antibodies in their sera (Table I). Fifty-eight per cent of them had titres of 1/80 or above. There was no difference in the incidence between the three races—Malay, Indian and Chinese. The same sera, including an extra one, were also tested for rubella haemagglutination inhibition antibodies (Thompson and Tobin, 1970). Seventy-one per cent of the subjects had titres of 1/20 or above, with the incidence of none-immunes apparently being higher in the Malay than in the Indian or Chinese (Table II).

The incidence of antibodies against CMV, HSV and EBV in 7-9 year-old school-children is given in Table III. CMV neutralizing antibody was detected in 88%, the neutralization test being used because many of the sera were found to be anticomplementary. Ninety-two per cent of the children had antibodies to EBV and 75% had antibodies to HSV.

DISCUSSION

The incidence of antibodies to CMV apparently differs in Asia and Africa to that found in North America and Europe. Studies in the latter areas have usually indicated less infant and childhood infection than in the East, but more adult infection. In the United Kingdom (Stern and Elek, 1965; Report, 1970) it was found that from 4-10% of individuals acquired antibodies during the first few years of life, a further 10% during primary schooling and a further 30-40% during adolescence and early adulthood, so that by the age of 35 years 50-60% of the population had acquired CMV antibody. This is similar to the pattern originally found in Washington DC by Rowe *et al* (1956) and by Mendez-Cashion *et al* (1963) in Puerto Rico.

In contrast, Alexander (1967) in Taiwan, showed that most infants were infected by the end of the first year of life, while in Egypt, Rowe (1960) found that 85% of children had antibody. Rowe has found high levels in children in certain western cities such as Paris and Helsinki (69%), and selected groups of elderly hospital patients in the north of England have also a higher incidence (89%) than the general population.

TABLE I

TITRE DISTRIBUTION BY RACE OF CYTOMEGALOVIRUS COMPLEMENT-FIXING ANTIBODY IN EXPECTANT MOTHERS

Titre	Malay	Indian	Chinese	Total
<10	—	—	1	1
10	—	—	1	1
15	1	—	2	3
20	4	—	5	9
30	—	—	2	2
40	1	1	7	9
60	2	3	12	17
80	8	6	43	57
TOTAL	16	10	73	99

TABLE II

TITRE DISTRIBUTION BY RACE OF RUBELLA HAEMAGGLUTINATION-INHIBITING ANTIBODIES IN 100 EXPECTANT MOTHERS

Titre	Malay	Indian	Chinese	Total
<20	7	2	20	29
20	—	—	—	—
40	—	1	11	12
80	3	1	5	9
160	1	2	13	16
320	3	1	9	13
640	2	1	13	16
1280	—	1	2	3
2560	—	1	1	2
% with Antibody	56	80	73	71

TABLE III

DISTRIBUTION OF ANTIBODIES TO CYTOMEGALOVIRUS, HERPES SIMPLEX AND EPSTEIN-BARR VIRUS IN SINGAPORE CHILDREN

Virus	No. Tested	No. Positive	% Positive
Cytomegalovirus (CMV)	48	42	88
Herpes simplex (HSV)	40	30	75
Epstein-Barr virus (EBV)	38	35	92

The high incidence of antibodies in Singapore in both school-children (88%) and expectant mothers (99%) suggests that most infection is acquired in infancy and early childhood as in Taiwan. Stern and Elek (1965) found that children from a boarding school had a much higher incidence than those attending day-school, and thought that close and prolonged contact was necessary for cross-infection. That CMV did not pass readily from person to person was suggested by Rowe from a study of CMV in day-nurseries and by a comparison of virus excreters and the incidence of antibody in Manchester school-children (Report, 1970).

It is most probable that the close family contacts amongst Asians in Singapore are responsible for the high incidence of CMV antibody in children. However, in Japan, Numazaki *et al* (1970) have suggested that infection in infancy is not due to horizontal spread of virus from infant to infant, but is acquired from the mother's cervix during birth, the latter's infection also having been acquired in infancy. The incidence of antibody found by these workers apparently did not increase with age as it did in Europe, nor did it reach the high incidence found in our Singapore sera. It is possible that infection in infancy in the East is partly maternally transmitted and partly acquired, while in Europe most of it is acquired. Also the incidence of cervical excreters of CMV is apparently much less in the United Kingdom (Report, 1970) than in Taiwan or Japan, and would account for only a very small number of infant infections.

Seventy-five per cent of the children had HSV neutralizing antibody, which is slightly higher than the 55% Smith *et al* (1967) found in 5-9 year-old children in Edinburgh, or the frequency for similar age-groups found recently in Manchester, and a little higher than was found in Japan by Yoshino *et al* (1962).

The percentage of positive sera (92%) against EBV in Singapore children is also higher than that which has been described elsewhere. Levy and Henle (1966) found that 50% of normal children in Uganda between the ages of 4 and 15 years were positive for FA antibody and this was similar to the incidence in American children of comparable age noted by Henle and Henle (1967). Henle *et al* (1968) noted that the frequency in New York and Cleveland was lower than in Philadelphia and ascribed this difference to socio-economic factors. In the South of England Pereira *et al* (1969) found antibody in 40% of 5-15 year-old children, while in Manchester 60% of children had acquired antibodies by the age of 10 years.

Besides their structural similarity HSV, EBV and CMV have much in common biologically. In all cases antibodies probably remain for life once they are acquired and the infecting agents may persist for a long time after primary infection. This is illustrated by the frequency of virus isolation from recurrent HSV lesions and by the high incidence of the transmission of EBV and CMV by blood transfusion, the virus presumably being present in the leucocytes.

It appears that Singapore children are infected with CMV, HSV and EBV at an earlier age than children in some western countries. The nature of Asian life ensures that there is close and prolonged contact between family members. This would enable these viruses to spread easily early in life and account for the high frequency of antibodies to them all in Singapore children. It is interesting though that rubella antibody in expectant mothers was found no more frequently in Singapore than in women in western countries (Report, 1967).

If socio-economic factors are the most important factors in CMV spread the incidence of antibody at different ages should slowly decrease, but if spread is longitudinal from mothers to infants the standard of living should have no effect.

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