Prevalence of Cytomegalovirus Antibodies in Thai Blood Donors*

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SUMMARY Antibodies to cytomegalovirus (CMV) were determined in Thai blood donors using the complement fixation (CF) test and enzyme-linked immunosorbent assay (ELISA). A total of 203 voluntary blood donors, 181 males and 22 females, who came to the Blood Bank at Siriraj Hospital during February 1985, were investigated. Their ages ranged from 17 to 53 years (mean 24.3 ± 6.9). Seventy-three out of 156 (46.8%) and 171 out of 203 (84.2%) sera were positive for CMV antibodies as detected by the CF test and ELISA respectively. The result of ELISA showed that 95.5 per cent of the female blood donors and 82.9 per cent of the males possessed CMV antibodies. No difference in the geometric mean titres of either sex was noted. The findings indicated that ELISA was more sensitive than the CF test for detecting CMV antibodies. The high percentage of CMV-seropositive blood donors indicates that CMV infection is common in this country. Therefore, it might be necessary to test blood donors for CMV antibodies when they are giving blood for use by certain patients, especially immunocompromised ones; the same observation applies with regard to organ donors before transplantation is carried out.

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Cytomegalovirus (CMV) is widely distributed among the human population throughout the world. Most CMV infections in healthy individuals are subclinical. However, clinical manifestations of such diseases are variable, particularly in certain high-risk patients. As with the other herpesviruses, primary infection with CMV is followed by life-long persistence of the latent virus. Three sites of CMV latency are leukocytes,[1,2] renal tissues[3,4] and genital organs.[5]

Post-transfusion CMV-mononucleosis was first described in patients receiving large amounts of blood during cardiac surgery.[6] Other prospective studies of blood recipients indicated that post-transfusion CMV infections occurred most frequently when seronegative recipients received seropositive blood, suggesting that CMV-seropositive blood donors were the source of the virus.[7] Transfusion-acquired CMV infection in infants seldom caused obvious illness.[8] However, it has been reported that the incidence rate of CMV infection is as high as 36 per cent in neonates after they have received blood transfusion(s) from seropositive and untested blood.[9]

The high incidence of CMV infection in patients receiving renal or bone marrow transplants is well recognised. Recipients of kidneys from CMV-seropositive donors face a significantly higher risk of infection and rejection than do recipients of kidneys from CMV-seronegative donors, particularly if the recipients are themselves seronegative.[3,4]

Bone marrow transplant patients whose donors possessed CMV antibodies suffered from CMV infection more frequently than those who received transplants from CMV-negative donors.[7,10] Moreover, when the donors were CMV-seropositive, CMV infection occurred in 25 per cent of the CMV-seronegative recipients as compared with 88 per cent in CMV-seropositive recipients after bone-marrow transplantation.[7] Thus in these CMV-seropositive patients, CMV was either reactivated by or transferred with the donor's marrow cells.

The purpose of this preliminary study was to determine the prevalence of antibodies to CMV in healthy Thai blood donors by using the complement fixation (CF)

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test and enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Blood samples

In February 1985, 203 serum specimens from healthy blood donors were obtained from the Blood Bank at Siriraj Hospital. The donors consisted of 181 males and 22 females, 92.5 per cent of whom resided in Bangkok and nearby areas. Their ages ranged from 17 to 53 years (mean 24.3 ± SD 6.9).

Complement fixation (CF) test

The test procedure was as described previously.[11] The sera were inactivated at 56°C for 30 minutes and diluted at a starting dilution of 1:8. Two units of CMV CF antigen and control antigen (Behring Institute, Germany) and two units of complement were used. The highest serum dilution giving 50 per cent haemolysis of sensitised sheep red blood cells was considered a positive result. Antibody titres were expressed as reciprocals of the positive serum dilutions. The criterion for the determination of CMV-seropositivity was an antibody titre of 8 or higher.

Enzyme-linked immunosorbent assay (ELISA)

Flat-bottom plates (Nunc, Denmark) were coated with 100 μl of CMV or control antigen in a 0.06 M carbonate buffer, pH 9.6. The optimal dilution of the coating antigens was 1:200, determined by checkerboard titration. The plates were incubated at 37°C for one hour and kept at 4°C overnight. Before running the test, the plates were washed three times with PBS containing 0.1% Tween 20 (PBS-T). Sera to be tested were diluted to 1:100 with PBS-T containing 0.5% bovine serum albumin (Sigma Chemical, U.S.A.). The plates were incubated at 37°C for two hours. After washing three times with PBS-T, 100 μl of a 1:1,000 dilution of rabbit antiserum to human IgG conjugated with alkaline phosphatase (Sigma Chemical, U.S.A.) were added to each well. After one hour of incubation at 37°C, the plates were again washed three times with PBS-T. To each well were added 100 μl of p-nitrophenyl phosphate in 10% diethanolamine buffer, pH 9.8. The reaction was stopped after 45 minutes of incubation by adding 50 μl of 3 N NaOH. The OD was read at 405 nm by an enzyme immunoassay (EIA) microplate reader (Titertek Multiskan, Flow Laboratories, U.S.A.). The reaction was indicated by the difference in absorbance of antigen and control antigen. The "cut off" value for positive reaction was $\geqslant 0.2$. The commercial test kit of Enzygnost®-Cytomegalie (Behring Institute, Germany) was used as a reference.

RESULTS

The distribution of CMV-seropositive blood donors according to age and sex is shown in Table 1. Seventy-three of 156 (46.8%) and 171 of 203 (84.2%) sera were positive for CMV antibodies by CF test and ELISA respectively. The prevalence rates of CMV infection as determined by both methods were slightly higher among female blood donors than male donors, 52.9% vs 46.0% and 95.5% vs 82.9% by CF test and ELISA respectively. CMV prevalence was found to increase with age, and 100% CMV seropositivity by ELISA was obtained in subjects who were over 40 years of age. (Forty-seven of the 203 (23.2%) sera could not be tested by CF because of anticomplementary activity (AC) in the sera.)

Table 2 shows the distribution of CMV antibody titres in the male and female donors. It was found that the geometric means of positive CF antibody titres in males and females were 19.7 and 18.7 respectively. There was no sex difference in GM antibody titres. The results of CMV antibodies detection using the CF test in comparison with ELISA are shown in Table 3. All 73 positive sera as obtained by both methods were comparable. Sixty-four (77.1%) negative serum

Table 1 CMV-seropositive blood donors by CF test and ELISA

Age-group (years)	CF test			ELISA		
	Male	Female	Total	Male	Female	Total
10 – 19	13/38† (34.2)	0/5 (0)	13/43 (30.2)	37/48 (77.1)	5/6 (83.3)	42/54 (77.8)
20 – 29	40/81 (49.4)	5/6 (83.3)	45/87 (51.7)	88/104 (84.6)	8/8 (100)	96/112 (85.7)
30 – 39	8/15 (53.3)	3/4 (75.0)	11/19 (57.9)	17/21 (81.0)	6/6 (100)	23/27 (85.2)
40 – 49	2/4 (50.0)	0/1	2/5 (40.0)	7/7 (100)	1/1	8/8 (100)
> 50	1/1	1/1	2/2 (100)	1/1	1/1	2/2 (100)
Total	64/139 (46.0)	9/17 (52.9)	73/156 (46.8)	150/181 (82.9)	21/22 (95.5)	171/203 (84.2)

Forty-seven of 203 sera showed anticomplementary activity (AC) in the CF test. † = No. positive/No. tested
Figures in parenthesis denote percentage

Table 2 Distribution of CF antibody titres

Sex	No. of donors at antibody titre of					GM
	<8	8	16	32	64	
Male	75	15	22	20	7	19.7
Female	8	2	4	2	1	18.7
Total	83	17	26	22	8	

GM = Geometric mean of the seropositive titres

Table 3 Sensitivity of CF test and ELISA

CF test Results	No. of donors	No. positive ELISA
Positive	73	73 (100%)
Negative	83	64 (77.1%)
AC	47	34 (72.3%)

samples and 34 (72.3%) of those showing AC in the CF test were positive by ELISA.

DISCUSSION

Our findings indicated that 46.8 per cent and 84.2 per cent of Thai blood donors were CMV-seropositive by CF test and ELISA respectively. The CF results obtained in this study were similar to those observed in Bangkok by Yamada *et al.*[12] Using ELISA technique, the result of 84.2 per cent CMV-seropositive Thai blood donors was higher when compared with the 32.5 per cent of donors in the 18-30-year age group and the 31.1 per cent of those in the 31-50-year age group as previously reported by Tantivanich *et al.*[13] However, a recent World Health Organisation survey of healthy blood donors from 26 countries revealed evidence of CMV antibodies in 40-50 per cent of persons from industrialised countries and in nearly 100 per cent of populations in developing countries.[14]

Our results showed a slightly higher prevalence rate of CMV infection among female blood donors than among male donors. Haldane *et al*[15] also reported a sex difference in the distribution of CMV, with females carrying CMV more frequently than males. However, there was no sex difference in GM antibody titres.

ELISA was more sensitive than the CF test for blood donor screening of CMV antibodies, as described in previous papers.[16,17] ELISA could detect a positive reaction in the sera with undetectable CF antibodies and eliminate the AC problem in the CF test. As high as 77.1 per cent of the sera negative by CF were found to be positive when ELISA was used. This suggested that some subjects with previous CMV infection had negative CF antibodies; therefore, the more sensitive ELISA should be performed to detect CMV antibodies. It is possible that those individuals with negative CF antibodies might harbour CMV. CMV-positive complement fixing antibodies were found in 52.9 per cent of non-pregnant women as compared with 74.3 per cent in pregnant women, as reported by Puthavatana *et al*[18] of the same institute. This supported the notion that pregnancy seems to increase the incidence of CMV infection as a result of CMV reactivation.

It is known that CMV-seropositive blood donors are associated with acquired CMV infection in post-transfusion syndrome and after renal or bone marrow transplantation. The high percentage of CMV-seropositive blood donors as noted in this study suggests that CMV infection is common in Thailand. Therefore, it is necessary to perform CMV antibody testing of blood donors for certain high-risk patients, especially immunocompromised ones, as well as of organ donors before transplantation. Such screening of donors would reduce the level of morbidity and mortality in patients at high-risk of contracting CMV infection after blood transfusion and also after renal or bone marrow transplantation.

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