PREVALENCE OF CYTOMEGALOVIRUS IN THAI BLOOD DONORS BY MONOCLONAL STAINING OF BLOOD LEUKOCYTES

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Abstract. Four hundred and forty-one blood and serum samples were collected during August to October 1998 from the blood donors at the blood bank of Rajvithi Hospital, Bangkok, Thailand. Their ages were varied between 18-55 years. All specimens were tested by immunostaining and ELISA methods. Forty-seven specimens (10.66%) gave positive results by immunostaining. Among these, 20 cases were seropositive and 27 cases were seronegative. The age group between 41-50 years had a high percentage of CMV infection as judged by the immunostaining method, more than the other age groups. By ELISA, 231 cases (52.38%) had positive IgG antibody to CMV, 42 cases (9.52%) were IgM antibody positive and 39 cases (8.84%) were positive for both IgG and IgM antibodies. The age groups between 36-40 years had a higher percentage of IgM antibody positives than the other age groups. Since the immunostaining method can detect early CMV infection, screening for the presence of antibodies alone is not enough to rule out CMV infection. Immunostaining along with ELISA detection of antibodies was useful for determining a decrease in CMV infection.

INTRODUCTION

Cytomegalovirus (CMV) can cause congenital infection and opportunistic infection in AIDS patients, and severe clinical problems in immunocompromised patients, *eg* during the first three months after renal transplantation (Betts, 1982; Macher *et al*, 1983). Transmission of the virus can occurr via sexual contact, congenital infection, organ transplantation and blood transfusion. In Thailand, CMV infection had been reported in normal pregnant women (Tantivanich *et al*, 1982), hospitality girls (Tantivanich *et al*, 1985), congenital neonates (Tantivanich *et al*, 1980) and in the normal population (Tantivanich *et al*, 1981). In order to minimize the rate of infection, it is necessary to avoid spreading of the virus, especially in the blood donors. Since spreading of the virus in the blood stream to various organs will occurr by blood leukocytes, early and accurate identification of CMV in leukocytes is important for treatment and for reducing the rate of infection.

In this study, immunostaining, which can be used to detect early CMV antigen, was introduced to assess the rate of CMV infection in Thai blood donors. The relationship for the presence of CMV antigen and the IgG and IgM antibodies was also performed, with a view to application to prevention of CMV infection in Thailand.

MATERIALS AND METHODS

Five ml EDTA treated blood were collected from 441 blood donors at the blood bank of Rajvithi Hospital, Bangkok, for detection of CMV in leukocytes by immunostaining. Sera were also collected from these blood donors for detection of IgG and IgM antibodies by enzyme-linked immunosorbent assay (ELISA). Their ages varied between 18-55 years.

Peripheral blood leukocytes isolation and immunostaining assay

Peripheral blood leukocytes from blood

donors were separated from EDTA- treated venous blood by density centrifugation. After washing twice in RPMI medium, 10 µl of cells were smeared on glass slides, air dried and fixed with acetone for 10 minutes at room temperature. The prepared PBL slides were incubated with monoclonal mouse anti-cytomegalovirus antibody AD 169 clone AAC 10 (Dako, Denmark) for 30 minutes at room temperature. After washing three times in PBS, 10 µl of 1:20 dilution of peroxidase-conjugate, rabbit anti-mouse immunoglobulins (Dako, Denmark)were added, and incubated another 30 minutes at room temperature. After that, the slides were washed again in PBS and the enzyme reaction was carried out with 3 amino, 9 ethycarbazol for 10 minutes followed by mild counterstaining with hematoxylin and mounting in glycerol-gelatin. The CMV infected and uninfected fibroblast cells were used as the positive and negative controls. The number of CMV under 25 x magnification greater than 5 CMV Ag^+ cells per slide were considered as a positive result.

Detection of IgG and IgM antibodies by ELISA

The CMV antigen was prepared in our laboratory by infecting human embryonic lung fibroblast cells with CMV strain AD 169 to give 3^+ CPE within 7 days. The infected cells were subjected to freezing and thawing 3 times in a dry-ice alcohol bath then centrifuged at 1,000 rpm for 10 minutes to remove the cell debris. The supernatant was sonicated at 10 KH/second for 3 seconds by using ultrasonic liquid process (Heat systems Inc, USA) then centrifiuged at 2,000 rpm for 10 minutes, a small aliquot of supernatant was retained as antigen. A dilution of 1:2,000 of the antigen was used to coat the plates.

The enzyme-linked immunosorbent assay (ELISA) for CMV was performed as described by Tantivanich (1980). The ELISA IgG and IgM antibodies were determined using peroxidase conjugated rabbit anti-human IgG specific for gamma chain and peroxidase conjugated rabbit anti-human IgM specific for Mu-chain (Dako). The dilution of the conjugates was 1:2,000 as recommended by the manufacturer. PPD (1,4 para-phenylene diamine dihydrochloride) was used as the substrate. The results were read at 492 nm in a multiscan spectrophotometer, and an OD equal to or higher than 2.50 was considered as positive.

RESULTS

Positive and negative CMV infections in PBL by the immunostaining method demonstrated in Fig 1. The positive cell staining showed dark brown color in the nucleus and red color in the cytoplasm while the negative results showed purple color both in the cytoplasm and nucleus. There was no cross-reaction of monoclonal antibody against CMV between CMV and herpesvirus type 6 and 7.

CMV was detected 47/441 (10.7%) in PBL blood donors by the immunostaining method. Among these, 20 (42.6%) were seropositive of which 14/20 (29.8%) had IgG antibody, 1/20 (2.1%) had IgM antibody, and 5/20 (10.6%) had both IgG and IgM antibodies. The remaining 27 cases gave seronegative results (Table 1).

Table 1
Comparison of the results between immunostaining positive and ELISA.

Immunostaining positive				
ELISA positive result			ELISA IgG and IgM negative	Total
IgG	IgM	IgG and IgM		
14 (29.8%)	1 (2.1%)	5 (10.6%)	27 (57.4%)	47

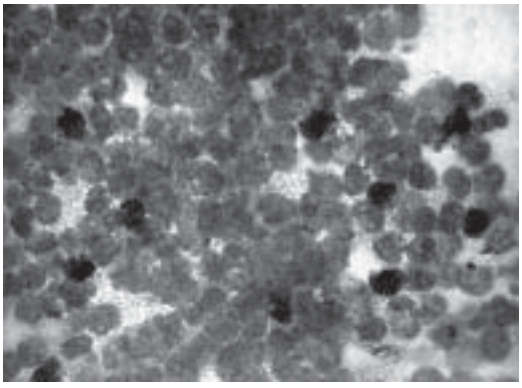

Fig 1a–Positive PBL staining under the light microscope with x40 magnification.

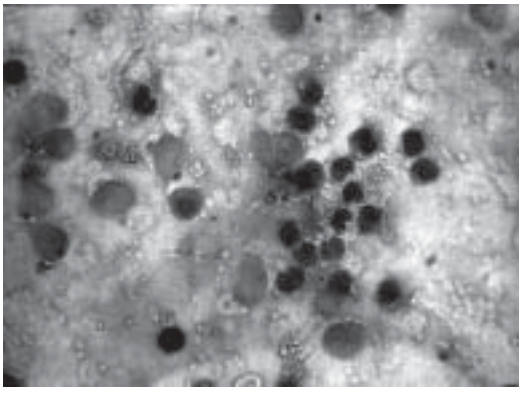

Fig 1b–Negative PBL staining under the light microscope with x40 magnification.

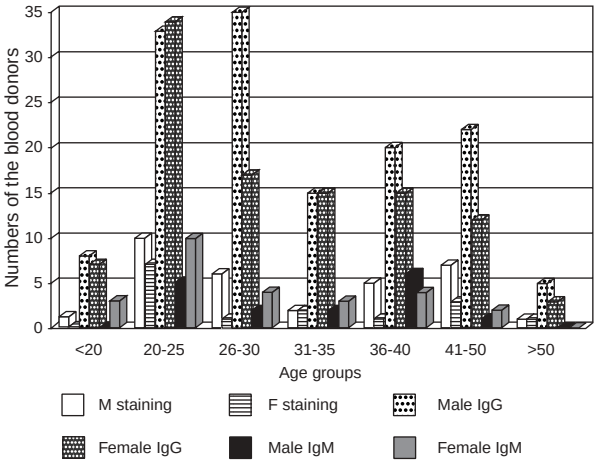

Fig 2–Age and sex distribution of the CMV positive blood donors by immunostaining, ELISA IgG and ELISA IgM method.

The results of IgG and IgM antibodies to CMV by ELISA according to sex are illustrated in Table 2. Two hundred and thirty-one cases (52.38%) were IgG serpositive. Among these, 128 (52.03%) were male and 103 (52.82%) were female. Forty-two cases (9.52%) were IgM seropositive. Sixteen (6.50%) of these cases were male and 26 cases (13.33%) were females. Of 39 cases (8.84%) who were IgG and IgM seropositive, 15 cases (6.10%) were male while 24 (12.03%) were female.

Distribution of CMV positive by immunostaining, IgG and IgM antibodies according to age and sex age demonstrated in Table 3 and Fig 2. Age group 6 (41-50years) had the highest percentage of positives while age group 1 (<20 years) had the lowest percentage by immunostaining. IgG antibody was found in both males and females of all age groups. Most of the IgM antibody positive were in group 5 (36-40 years) and group 2 (20-25 years) respectively. Females were prone to have more IgM antibody than male in almost all age groups except in age group 5 (36-40 years).

The relationships of CMV infection demonstrated by immunostaining to the presence of anti-CMV antibodies titers are demonstrated in Table 4 and Table 5.

DISCUSSION

Cytomegalovirus infections can be found all over the world. The rate of infection varies in different population and different age groups (Numasaki *et al*, 1970). In this study, the percentage of blood donors with CMV antibodies was quite high (70.75%) when compared to the previous study in 1981 (47%) (Tantivanich *et al*, 1981), but very close to the result in 1998 (71.8%) (Tantivanich *et al*, 1998). The reason for this may be the different time and different groups of the population studied. Females are prone to have more CMV antibodies than males (Table 2). The age group 2 (20-25 years) had higher numbers of CMV infection by immunostaining than other groups

Table 2
Distribution of CMV infection by immunostaining, IgG and IgM by ELISA according to sex.

Sex	Total	Positive test (%)			
		Immunostaining	ELISA IgG	ELISA IgM	ELISA IgG and IgM
Male	246	32 (13.01%)	128 (52.03%)	16 (6.50%)	15 (6.10%)
Female	195	15 (7.69%)	103 (52.82%)	26 (13.33%)	24 (12.30%)
Total	441	47 (10.66%)	231 (52.38%)	42 (9.52%)	39 (8.84%)

Table 3
Distribution of CMV positive cases according to age groups.

Group No.	Age group (year)	Total case	Immunostaining		ELISA IgG		ELISA IgM		ELISA IgG and IgM	
			positive case	% positive	positive case	% positive	positive case	% positive	positive case	% positive
1	<20	27	1	3.70	15	55.56	3	11.11	3	11.11
2	20-25	122	17	13.93	67	54.92	16	13.11	14	14.47
3	26-30	102	7	6.68	52	50.98	6	5.88	6	5.88
4	31-35	60	4	6.67	31	51.67	5	8.33	5	8.33
5	36-40	66	6	9.09	35	53.03	9	13.64	8	12.12
6	41-50	50	10	20.00	23	46.01	3	6.00	3	6.00
7	>50	14	2	14.29	8	57.14	0	0.00	0	0.00
Tatal		441	47	10.66	231	53.38	42	9.52	39	8.84

Table 4
Relationship of CMV infection by immunostaining and the presence of CMV antibody by ELISA.

ELISA	Antibody titer	Immunostaining		Tatal
		positive (%)	negative (%)	
IgG positive	1:80 - 1:320	12 (7.8)	142 (92.2)	154
	1:640 - 1:2,560	2 (5.3)	36 (94.7)	38
IgM positive	1:80 - 1:320	1 (33.33)	2 (66.67)	3
	1:640 - 1:2,560	0	0	0
IgG and IgM positive	1:80 - 1:2,560	5 (12.8)	34 (87.2)	39
IgG and IgM negative		27 (13.0)	180 (87.0)	207
Total		47	394	441

Table 5
Relationship of CMV infection by immunostaining and the presence of CMV antibodies by ELISA.

Method	IgG positive	IgM positive	IgG, IgM positive	IgG, IgM negative
Immunostaining positive	Reactivation or reinfection	Active CMV infection	Active CMV infection reactivation or reinfection	Early CMV infection 1º infection
Immunostaining negative	Past infection	Recent infection	Reinfection and late stage of infection	No infection

(Fig 2). The presence of IgM antibody in this age group correlated well with the result by the immunostaining method which indicated active CMV infection. For the other age groups, the results of IgM antibody did not correlate with the results by immunostaining methods. In age groups 1,4 and 5, the percentage of IgM antibody was higher with immunostaining, while in age groups 3, 6 and 7 the percentages of IgM antibody were lower by immunostaining (Table 3).

Detection of CMV infection by the immunostaining method is helpful for early diagnosis, especially for blood transfusion and organ transplantation. This method can detect the presence of CMV in leukocytes very early during the course of an active infection and before seroconversion or positive virus cultures (Saltzman *et al*, 1988). The presence of CMV in leukocytes with IgG antibody indicated that the blood donors had been infected with CMV which may result from reinfection or reactivation. The absence of CMV in leukocytes of individuals with IgG antibody indicates past infection in the blood donors before the time of study. The presence of CMV in leukocytes in blood donors without IgG or IgM antibodies indicates early CMV infection or primary infection since we could detect the virus prior to the presence of antibody.

The presence of IgM antibody indicates active infection. In this study, the donors who had positive immunostaining results with the presence of IgM antibody had active infection since we could detect both the antigen and antibody at the same time. This type of infection could be either primary or recurrent in nature. The blood donors who had IgM antibody with negative immunostaining may reflect recent infection by CMV or cross reaction with herpes simplex type 6 or type 7 (Osman *et al*, 1997) since the levels of IgM antibody titer were not high (< 1:320) as demonstrated in Table 4.

The presence of both IgM and IgG antibodies with positive immunostaining also indicated active CMV infection, reactivation or reinfection. The absence of CMV in leukocytes with the presence of both IgG and IgM antibodies indicated reactivation and progressive infection similar to the presence of IgM alone as mentioned above. No infection was found in the group of blood donors who gave negative results by both immunostaining and antibody detection.

From the results of this study, it can be concluded that CMV infection can be found in the normal Thai population in high percentage and the immunostaining method is quite useful for screening CMV in blood donors before giving blood to patients in addition to using the high efficiency leukocyte filter (De Witte *et al*, 1990, Sayer *et al*, 1992). Since the immunostaining technique can detect early antigen of CMV prior to the presence of antibodies, screening for the presence of antibodies alone is not enough to exclude CMV infection. Immunostaining for detection of CMV in leukocytes along with detection of antibodies should be introduced to every blood bank in order to reduce CMV transmission rate and to reduce congenital infection.

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