RESEARCH NOTE

PREVALENCE OF HUMAN CYTOMEGALOVIRUS (HCMV) GB GENOTYPES IN THAI PATIENTS

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Abstract. Human cytomegalovirus (HCMV) infection can cause asymptomatic to symptomic diseases leading to morbidity and mortality especially in immunocompromized patients. One factor of the difference in clinical outcome is the distinction of HCMV strain. As HCMV glycoprotein (g)B plays an important role in viral entry and neutralizing antibody induction, HCMV gB genotypes were determined in 161 clinical specimens containing HCMV-DNA obtained from patients at King Chulalongkorn Memorial Hospital, Bangkok, Thailand during the year 2000 and 2004. Of the 113 (70%) samples that were able to be genotyped, mixed gB genotype was demonstrated in 35%, followed by gB1 (33%), gB3 (15%), gB2 (11%), and untyped (7%); gB4 was not detected. The distribution of HCMV gB genotypes between genders was not significantly different. Mixed gB genotype (35%) was found in HIV- infected patients.

INTRODUCTION

Human cytomegalovirus (HCMV) is a large envelope virus containing double stranded DNA belonging to beta herpesvirinae, a subfamily of herpesviridae. Most individuals become infected with HCMV early in life, and depending on the geographic location, between 60% and 100% of adults are carriers of the virus (Britt and Alford, 1996). Generally, HCMV infection in healthy persons is usually asymptomatic or mild. Reactivation is clinically silent in immunocompetent individuals, although infectious virus might be shaded in various organ excretions (Dworsky *et al,* 1983; Collier *et al,* 1995). In immunocompromized patients with AIDS or transplant recipients HCMV can lead to serious manifestation such as fever, leukopenia, hepatitis, nephritis, pneumonia, arthralgias, esophagitis, gastritis, enteritis, colitis, encephalopathy or encephalitis and retinitis, and in some cases the disease may be fatal (Jacobson and Mills, 1988; Ljungman *et al,* 2002).

There are many attempts to study factors influencing the variation of HCMV diseases, both in host and virus itself. Several reports have suggested an association of different gB genotypes with pathology (Fries *et al,* 1994; Rasmussen *et al,* 1997; Torok-Storb *et al,* 1997), as gB is considered to be a multifunctional envelope component responsible for virion entry, and cell to cell spread, and is the major target for neutralizing antibodies (Rasmussen *et al,* 1988; Navarro *et al,* 1993). HCMV gB genotyping is based on the highly variable region around the proteolytic cleavage site, and 4 gB genotypes have been observed (Chou and Dennison, 1991). Although

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gB genotypes were demonstrated in some studies to correlate with the clinical outcome of HCMV infection (Fries *et al,* 1994; Rasmussen *et al,* 1997; Torok-Storb *et al,* 1997; Coaquette *et al,* 2004), no association was found in other studies (Chern *et al,* 1998; Gilbert *et al,* 1999; Aquino and Figueiredo, 2000; Arista *et al,* 2003).

In Thailand, most of the Thai population have been infected with HCMV early in their life (Pancharoen *et al,* 1998; Tantivanich *et al,* 1999; Likitnukul *et al,* 2003) with seroprevalence of 70.7 to 100% in adult blood donors and pregnant women (Urwijitaroon *et al,* 1993; Bhattarakosol *et al,* 1998; Amarapal *et al,* 2001). Nevertheless, no information on gB genotypes among Thai patients is available. In this present study, HCMV gB genotypes in clinical specimens were determined.

MATERIALS AND METHODS

Clinical specimens

A total of 161 HCMV-DNA positive specimens detected by polymerase chain reaction (PCR) from 128 patients were obtained from Virology Unit, King Chulalongkorn Memorial Hospital, Bangkok, from the year 2000 to 2004. Clinical specimens included 110 plasma, 33 white blood cells (WBC), 5 cerebrospinal fluid (CSF), 4 bronchoalveolar lavage (BAL), 2 throat washings, 1 tissue, 1 sigmoid, 1 duodenum, 1 endotracheal tube (ETT) secretion, 1 urine, 1 liver biopsy and 1 lung biopsy.

HCMV gB genotyping

For DNA extraction, all samples were processed using QIAamp® DNA Mini Kit. HCMV gB genotype was determined using nested PCR and restriction fragment length polymorphism (RFLP) analysis applied as described by Chou and Dennison (1991). The first round of amplification was performed with primers gB1043 and gB1724 and the second round of PCR was conducted with internal primers gB1319 and gB1604. The amplified gB products were digested with restriction enzyme *Hin*fI and *Rsa*I (Invitrogen, USA) and separated by electrophoresis at 80 volts in 7% polyacrylamide gel. From the results of this analysis 4 gB genotypes could be distinguished by their different patterns of fragment lengths (Chou and Dennison, 1991). If the results showed different patterns from these four genotypes, it was classified as "untype" (UT). Mixed gB genotypes were able to be identified, but mixed infection of gB1+3, gB2+4 or more than 2 gB genotypes could not be identified due to their identified pattern. Therefore, we classified these as "mixed unclassified types" (MUT).

Statistical analysis

ANOVA and chi-square test were performed for analyzing statistically significant difference.

RESULTS

A total of 161 clinical specimens, previously known to be positive HCMV-DNA, from 128 patients were directly genotyped by PCR-RFLP. HCMV gB genotyping was successful in 113 (70%) samples from 96 patients (48 females and 48 males). Thirty-one of 96 patients were HIV- infected patients and 13 were transplant recipients. Mixed gB genotype was most frequently found (39 samples, 35%), followed by gB1 (37, 33%), gB3 (17, 15%), gB2 (12, 11%), and UT (8, 7%). No gB4 was observed. Of these 8 UT samples, 2 patterns (UT1 and UT2) were obtained (Fig 1). In addition, 8 patterns of mixed gB genotypes were found (Fig 2), including gB1+2 (6 samples, 15%), gB1+4 (2, 5%), gB2+3 (12, 31%), gB3+4 (4, 10%), gB1+2+UT (1, 2%), gB2+3+UT (1, 2%), gB3+4+UT (1, 2%), and MUT (12, 31%). Five out of 96 patients demonstrated different patterns of gB genotypes in samples collected at different times (Table 1). No significant difference in gB genotype distribution between

Table 1
HCMV gB genotypes in different clinical samples of the same person.

Number	Code	Date	Sample	gB genotype
P1	6F	14-8-03	Plasma	1+2
	2E	29-8-03	Throat washing	2
	3E	8-9-03	BAL	2
P2	5F	15-7-02	Plasma	3
	9I	12-13-02	Plasma	2+3
P3	2I	21-1-01	Plasma	1+4
	8F	21-2-04	Plasma	1+2
	3I	23-3-04	Plasma	MUT
P4	6B-2	24-4-03	Plasma	2
	4H	9-7-03	Plasma	1+2+untype
P5	10H-2	27-8-04	Plasma	3
	2I-2	3-9-04	Liver biopsy	2+3

BAL = Bronchoalveolar lavage

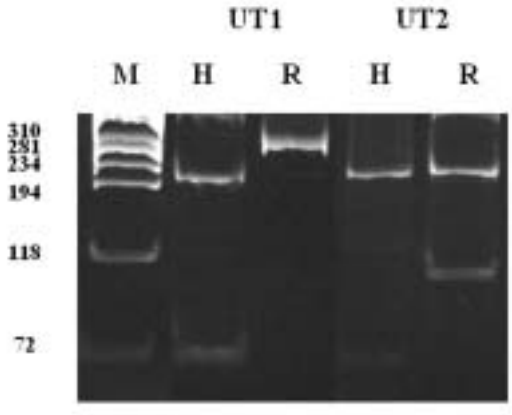

Fig 1–Restriction digestion pattern of the untyped gB genotypes (UT). M, DNA molecular sizes marker (øx174 *Hae*III digest, bp); H, *Hin*fI digestion; R, *Rsa*I digestion.

sex was observed (chi-square test, p-value = 1).

Forty-four samples were from 2 groups of immunocompromized patients, 13 transplant recipients and 31 HIV- infected patients. Only 1 sample of gB2 genotype was found in transplant cases, whilst UT was detected only in HIV- infected patients. gB1 (62%) was predominantly found in transplant patients, whereas mixed gB genotypes (35%) was the most dominant in HIV patients. Although mixed infection in HIV patients was higher than transplant patients, the distribution of gB genotypes between these 2 groups was not statistically different (chi-square test, p-value = 0.99).

DISCUSSION

In the present study, HCMV gB genotype was able to be determined directly in 70% of clinical specimens. Samples that could not be detected might be due to the small amount of HCMV in the samples. Three unique gB genotypes (gB1, gB2 and gB3) as well as untype (UT) were observed. Mixed gB genotypes with different genotype combinations were also found. No gB4 was detected in this study. Our data were similar to other studies showing that HCMV infection with gB1, gB2 and gB3 was relatively common and that infection with gB4 was uncommon (Meyer-Konig *et al,* 1998; Carraro and Granato, 2003; Coaquette *et al,* 2004). MUT and gB2+3 (31% each) were the most prevalent among our Thai

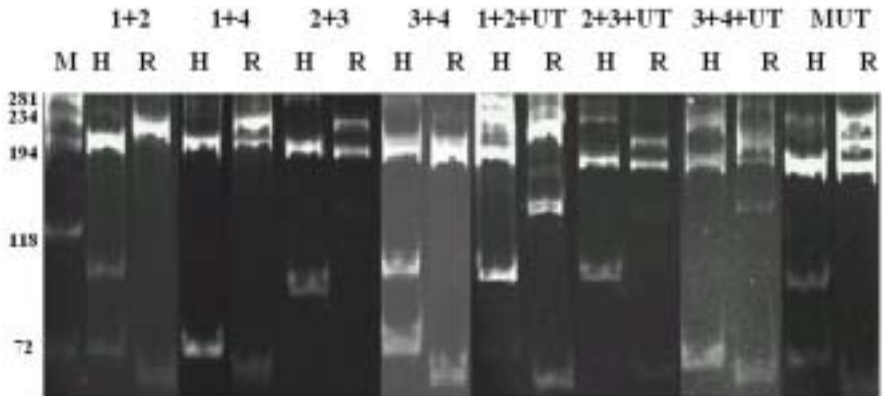

Fig 2–Restriction digestion pattern of mixed HCMV gB genotypes. M, DNA molecular sizes marker (øx174 *Hae*III digest, bp); H, *Hin*fI digestion; R, *Rsa*I digestion; UT, untype gB genotype; MUT, mixed unclassified type.

patients but other studies found a greater predominance of other combination of gB types such as gB1+3 (Aquino and Figueiredo, 2000; Coaquette *et al,* 2004). The differences in genotype frequency may, in part, be due to variation in study population as well as in the geographical distribution of HCMV genotype. Interestingly, 2 of the untype HCMV gB genotypes (UT1 and UT2) were identified in this present study; UT2 was similar to a previous report (Rasmussen *et al,* 1997), but UT1 has never been reported elsewhere.

A high proportion of patients (more than 90%) were reported to be infected with only one gB genotype (Fries *et al,* 1994; Rasmussen *et al,* 1997; Torok-Storb *et al,* 1997; Chern *et al,* 1998; Meyer-Konig *et al,* 1998; Aquino and Figueiredo, 2000; Arista *et al,* 2003; Carraro and Granato, 2003; Coaquette *et al,* 2004), in agreement to our results, 66%. Different patterns of gB genotype were observed among samples collecting at different time from 5 HCMV- infected patients (Table 1). This phenomenon may be due to reinfection of new strains, viral immune evasion from neutralizing antibody or recombination of existing strain with the new viral strain.

As HCMV is associated with various types of diseases, positive clinical specimens may be obtained from different sites of the body. The prevalence of gB genotypes differed if samples were obtained from different sites as well as the presence of more than a single gB genotype in different samples of the same patient (Table 1). This observation was similar to other reports (Rasmussen *et al,* 1997; Meyer-Konig *et al,* 1998; Aquino and Figueiredo, 2000).

Among HIV- infected and transplant patients, gB1 (62%) was the most common type among transplant patients, while mixed gB genotype infection was dominant in HIV- infected patients. Although many observations reported high prevalence of gB2 in HIV- infected patients (Rasmussen *et al,* 1997; Chern *et al,* 1998; Gilbert *et al,* 1999; Arista *et al,* 2003), no gB2 was detected in our samples. However, high gB1 prevalence in transplant recipients was the same as in other studies (Fries *et al,* 1994; Rasmussen *et al,* 1997; Torok-Storb *et al,* 1997). The high prevalence of mixed HCMV infection in HIV- infected patients was not unexpected since the immune response of these patients is very poor and reactivation or reinfection of HCMV could of-

ten repeatedly occur.

In summary, this study demonstrated that a single HCMV gB genotype infection was predominantly found (66%) and gB1 was the most prevalent. Prevalence of mixed HCMV infection was high in HIV patients.

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