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New Phenomenon

Association of human leukocyte antigen E polymorphism with human cytomegalovirus reactivation in Chinese burn patients

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The seroprevalence of human cytomegalovirus (HCMV) in adults increased steadily from 55% in developed countries to over 90% in developing countries like China [1]. As all herpesviruses, HCMV establishes lifelong latency after primary infection. In immunocompetent individuals, host immune responses prevent the development of overt HCMV diseases. However, in immunocompromised people who suffer from burn injuries, HCMV reactivation has been shown to lead to significant diseases with considerable morbidity and mortality [2–4]. Recently, increasing evidence has suggested that HCMV reactivation might have been considerably underestimated in burn patients [5,6]. Review of the available literature identifies >50% of HCMV antibody-positive burn patients may reactivate this virus [5,6]. Although the exact mechanisms of HCMV reactivation are still not clearly understood, the immune system and host genetics are thought to be the non-behavioral factors determining the acquisition of a reactivation.

Numerous studies have suggested that human leukocyte antigen (HLA)-E plays an important role in regulating anti-HCMV immunity [7–11]. It is reported that in HCMV-infected cells, classical major histocompatibility (MHC) class I molecules are down-regulated, but the MHC class Ib molecule HLA-E is normally expressed or even overexpressed on the cell surface [8–10]. Because the possible role of HLA-E in the HCMV infection, it is important to understand the biological significance of different alleles of the HLA-E. In this study, we assessed whether the functional polymorphism of the HLA-E locus influences the susceptibility or resistance to HCMV reactivation in patients suffering from burn injuries.

A total of 160 burn patients, who met the following inclusion criteria, were included: (i) be able to give informed consent (either from patient or relatives); (ii) total burn surface area (TBSA) >15% [5]; (iii) expected survival >72 h; (iv) HCMV-IgG seropositive within 24 h from admission; (v) no known or suspected underlying immunodeficiency (solid organ or hematopoietic stem cell transplant, human immunodeficiency virus infection, congenital immunodeficiency, and receipt of immunosuppressive agents). Characteristics of these patients such as age, gender, %TBSA, and inhalation injury are listed in **Table 1**. Serum samples were collected once or twice a week and stored at −20°C for subsequent HCMV polymerase chain reaction (PCR) analysis or HLA-E genotyping. Patients were prospectively followed until death or hospital discharge. The study was approved by the Medical Ethics Committee of Nantong University (Wuxi, China).

Enzyme linked immunosorbent assay of anti-HCMV IgG antibody was performed with a commercially available HCMV diagnostic kit (Beier Bioengineering, Beijing, China) according to the manufacturer's instructions. HCMV DNA testing was carried out using the *Artus* CMV TM PCR kit (Qiagen, Hilden, Germany) on an ABI PRISM® 7900HT sequence detection system (Applied Biosystems, Carlsbad, USA). A standard curve was obtained from the quantitation standard (QS) CMV DNA positive controls (CMV TM QS 1–4) provided by the manufacturer.

HLA-E genotyping was determined by restriction fragment length polymorphism (RFLP) system as described previously [12]. Briefly, the known HLA-E alleles differ at only one amino acid position, with either an arginine (the HLA-E*0101) or a glycine (the HLA-E*0103) at position 107. Thus, a forward primer with a deliberate mismatch (underlined) introduced at the second position from 3′-terminus (5′-GGCTGCGAGCTGGGGCCCGCC-3′) and a reverse primer (5′-AGCCCTGTGGACCCTCTT-3′) were designed. After a small sized PCR product of ~270 bp (confirmed by nucleotide sequencing) was amplified by PCR analysis, the presence of the HLA-E*0103 allele was identified by the presence of a restriction site of *Hpa*II enzyme (created in combination with the mismatch), which cuts the HLA-E*0103 allele into two fragments, 249 and 20 bp. However, the HLA-E*0101 allele cannot be cut by *Hpa*II shown as a band at 270 bp. The resulting fragments can be

separated according to their lengths by agarose gel electrophoresis (**Fig. 1**). All of the statistics were performed by using the SPSS software (version 19.0; SPSS Inc., Chicago, USA). Quantitative data were presented as median and range, while qualitative data were presented as number and percentage. Allelic frequencies were compared between different groups either by χ^2 test or Fisher's exact test when needed. The corrected P values (P_c) were obtained by multiplying P values (two-tailed) by the number of alleles tested. Significance level was set at $P = 0.05$.

Table 1 Characteristics of burn patients with and without HCMV reactivation

	HCMV reactivation ($n = 108$)	Number of HCMV reactivation ($n = 52$)
Mean age	32.3 ± 10.1	38.5 ± 9.5
Gender ratio (M/F)	66 / 42	32 / 20
%TBSA	35.3 ± 12.2	32.7 ± 15.3
Inhalation injury [*n* (%)]	28 (25.9%)	17 (32.7%)
Severe sepsis [*n* (%)]	43 (39.8%)	13 (25.0%)

M, male; F, female.

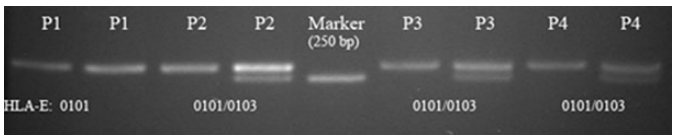

Figure 1 ***HLA-E*** **gene polymorphism by RFLP using** ***Hpa*****II restriction enzyme** After restriction enzyme *Hpa*II digestion, the PCR products from the same patient (pre-digested and post-digested) were simultaneously detected in 2% agarose gel. For each sample, the left side was the pre-digested aliquot and the right side was post-digested. The presence of one band at 270 bp was interpreted as the HLA-E*0101 allele, the presence of one band at 249 bp was interpreted as HLA-E*0103 (20 bp was not visible in the 2% gel), and the presence of two bands at 270 and 249 bp was interpreted as HLA-E*0101/*0103 alleles. The fifth lane shows a DNA molecular weight marker at 250 bp.

The results showed that among the 160 burn patients, 108 patients experienced HCMV reactivation. Of note, allele HLA-E*0101 was found to be higher in patients with HCMV reactivation group, and the difference was statistically significant ($P = 0.018$, $P_c = 0.036$) (**Table 2**). Compared with the genotypic distribution of HLA-E alleles, the HLA-E*0101/E*0101 genotype was more prevalent among groups with HCMV reactivation than their counterparts ($P = 0.007$, $P_c = 0.021$) (**Table 2**).

Although the HLA-E*0101 and *0103 alleles differed only at the amino position 107, both of the two alleles had obvious differences in peptide affinity and cell surface expression [13]. In contrast to the HLA-E*0101 allele, the HLA-E*0103 allele was more thermally stable than HLA-E*0101 evidenced by a higher surface expression of the peptides/HLA-E*0103 complex [13]. It was therefore confirmed that subtle but significant difference between the two alleles may influence both host innate and adaptive immunity [14]. The current data allow us to postulate that the association between a homozygous HLA-E*0101 allele and HCMV reactivation in burn patients may reflect the less-efficient presentation of virus-derived peptides resulting in diminishing the capacity of mounting efficient $CD8^+$ T-cell response, in conditions where the threshold for reactivations has been lowered because of immunocompromised diseases such as burn.

Taken together, our results revealed that the HLA-E locus may either directly or by a linked locus play a role in mediating HCMV reactivation risk in Chinese burn patients, which are of importance for future studies of more aggressive antiviral treatments. Finally, it has to be mentioned that due to the limitation of the restricted number of examined patients, our findings may require confirmation on a much larger cohort in the future. Also, the exact mechanisms for this association remain to be established.

Table 2 HLA-E allele and genotype frequencies among patients with and without HCMV reactivation

HLA-E polymorphism	HCMV reactivation ($n = 108$)	No HCMV reactivation ($n = 52$)	χ^2	P	P_C
Alleles					
*0101	105 (48.61%)	36 (34.62%)	5.579	0.018	0.036
*0103	111 (51.39%)	68 (65.38%)			
Genotypes					
0101/0101	38 (35.19%)	8 (15.38%)	7.347	0.007	0.021
0101/0103	29 (26.85%)	20 (38.46%)	2.227	NS	NS
0103/0103	41 (37.96%)	24 (46.15%)	–	NS	NS

Allele and genotype frequencies were compared between the two groups of patients (HCMV reactivation vs. no HCMV reactivation) either by χ^2 test or Fisher's exact test when needed. The corrected P values (P_c) were obtained by multiplying P values (two-tailed) by the number of alleles tested.

NS, not significant.

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