Human Immunology 76 (2015) 161–165

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

HLA-E polymorphism and clinical outcome after allogeneic hematopoietic stem cell transplantation in Egyptian patients

Ghada I. Mossallam [a,*], Raafat Abdel Fattah [b], Alaa El-Haddad [c], Hossam K. Mahmoud [b]

[a] Bone Marrow Transplantation Laboratory Unit, National Cancer Institute, Cairo University, Cairo, Egypt
[b] Department of Medical Oncology, National Cancer Institute, Cairo University, Cairo, Egypt
[c] Department of Pediatric Oncology, National Cancer Institute, Cairo University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 20 June 2014
Accepted 15 December 2014
Available online 25 December 2014

Keywords:
HLA-E
Hematopoietic stem cell transplantation
Graft versus leukemia (GVL)
Relapse

ABSTRACT

Human leukocyte antigen-E (HLA)-E in a non-classical major histocompatibility complex (MHC) class I (Ib) molecule. HLA-E-peptide complex acts as a ligand for natural killer (NK) cells and CD8+ T lymphocytes playing a dual role in natural and acquired immune responses. The difference in expression levels between HLA-E alleles was suggested to have impact on transplantation outcome. The aim of the study is to evaluate the clinical effect of HLA-E alleles on transplantation in a group of Egyptian patients. HLA-E genotyping was analyzed in eighty-eight recipients of stem cell transplantation using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). HLA-E*01:03 allele showed a trend towards lower cumulative incidence of relapse at 2 years compared to homozygous HLA-E*01:01 genotype (8% versus 21.5%, $p = 0.09$, HR: 0.30, 95% CI: 0.91–1.69). HLA-E was the only factor showing near significant association with relapse incidence. HLA-E polymorphism did not affect the cumulative incidence of acute GVHD grades II-IV at 100 days, the 2-year cumulative incidence of extensive chronic GVHD, transplant related mortality (TRM) or overall survival (OS). *Conclusion:* the suggested association of HLA-E polymorphism with reduced risk of relapse needs verification in a larger cohort. However, its proposed role in GVL helps better understanding of alloreactivity of T cells and NK cells and their implication in immunotherapy post allogeneic hematopoietic stem cell transplantation.

1. Introduction

Allogeneic hematopoietic stem-cell transplantation (AHSCT) is a curative therapy for many hematologic malignancies. Post transplantation complications are mainly related to the development of graft versus host disease (GVHD), infection and relapse. Human leukocyte antigen-E (HLA-E) in a non-classical major histocompatibility complex (MHC) class I (Ib) molecule characterized by limited polymorphism and lower cell surface expression compared to classical Class Ia molecule [1,2]. It is an immunomodulatory molecule that can function as both immuno-tolerogenic and immuno-activating molecule and plays a dual role in natural and acquired immune responses [3,4]. The HLA-E-peptide complex can act as a ligand for the CD94/NKG2 receptors expressed on the surface of natural killer (NK) cells and represents a restriction element for TCR receptor of CD8+ T cells [5–7]. HLA-E molecule binds and presents a restricted set of peptides derived from self or foreign proteins [8]. It binds nonamer peptides derived from signal sequences of other HLA class I molecules including HLA-A, -B,-C, and -G [4,9]. Its surface expression is regulated by the availability of these peptides [10]. HLA-E is expressed on the extravillous trophoblasts and mediates maternal tolerance to semiallogeneic fetal graft [11]. HLA-E can also bind viruses [12,13], bacteria [14], tumors [4], stress-related peptides [7], and self-antigens [15]. Recognition by CD94/NKG2 is governed by the sequence of peptide bound to HLA-E [16]; CD94/NKG2A functions as an inhibitory receptor, while CD94/NKG2C functions as an activating receptor [17]. HLA-E mediates lysis of targets by CD8+ cytotoxic T cell (CTL) including NK–CTL and regulates adaptive immune response through CD8+ suppressive T cells [5–7,18].

Two nonsynonymous functional alleles of HLA-E have been found, E*01:01 and E*01:03 [19]. An arginine at position 107 located within the α2 domain of the HLA-E heavy chain in HLA-E^{107A} (HLA-E*01:01) is replaced by a glycine in HLA-E^{107G} (HLA-E*01:03) [20]. The functional differences between the HLA-E alleles are related to the relative peptide affinity and cell surface

* Corresponding author at: National Cancer Institute, Cairo University, Fom El-Khalig Square, Kasr El-Aini St., 11796 Cairo, Egypt.
E-mail address: ghadamossallam@hotmail.com (G.I. Mossallam).

http://dx.doi.org/10.1016/j.humimm.2014.12.017
0198-8859/

expression, with the HLA-E*01:03 allele expressed at a higher levels than *01:01, due to its higher affinity to peptides and therefore higher surface stability [4,10].

HLA-E polymorphism has been associated with infection, cancer, recurrent spontaneous abortion, autoimmune diseases, and transplantation outcome [4,11,13–15,21]. HLA-E polymorphism was found to affect HSCT outcome; reports pointed to the association of HLA-E*01:03 genotype with protection from graft versus host disease and relapse as well as better survival, while HLA-E*01:01 homozygosity was associated with risk of infection post AHSCT [21–27]. The aim of the study is to evaluate the clinical impact of HLA-E alleles on transplantation in Egyptian patients after AHSCT from HLA-matched sibling donors.

2. Patients and methods

2.1. Patients

The cohort consisted of 88 recipients transplanted from their HLA-identical siblings at Bone Marrow Transplantation (BMT) Unit, National Cancer Institute, Cairo University and at Nasser Institute between 2007 and 2010. Informed consent was obtained. They were 51 males and 37 females. Their median age was 30 years, range (2–48). They suffered from: acute myeloid leukemia (AML) 52/88(59.1%), chronic myeloid leukemia (CML) 21/88(23.9%) and myelodysplastic syndrome (MDS) 15/88(17%). Patients' characteristics are mentioned in Table 1. Donor selection was done using serologic method (BIO-Rad) for HLA class I typing, while sequence specific oligonucleotide probe (SSOP) method (Inno-Lipa HLA-DRB1 plus) was used for class II typing. HLA-E genotyping was performed for recipients using polymerase chain reaction-restricted fragment length polymorphism.

Conditioning regimens and GVHD prophylaxis

Conditioning regimens included Flu/Bu: Fludarabine 30 mg/m^2/d IV daily for 4 days (D-10, D-9,D-4 and D-2) and Busulphan 4 mg/kg p.o. daily for 4 days (D-8 to D-5); Flu/Alk: Fludarabine 30 mg/m^2/d IV daily for 4 days (D-7 to D-4) and Alkeran (Melphalan) 70 mg/m^2/d IV for 2 days (D-3 and D-2); Bu/cy: Busulphan 4 mg/kg p.o. daily for 4 days (D-7 to D-4) and Cyclophosphamide 60 mg/kg/d IV for 2 days (D-3 and D-2) given with 2-mercaptoethane sulfonate sodium (MESNA).

GVHD prophylaxis was given by Cyclosporin-A (CSA) 3 mg/kg IV from day −1 to be changed to oral form (3 mg/kg) once the patient can swallow for 9–12 months post-transplant. GVHD prophylaxis included also short course IV methotrexate (15 mg/m^2 day 1 and 10 mg/m^2 days 3 and 6) with leucovorin rescue and mycophenolate mofetil (MMF) 1000 mg twice daily PO.

2.2. HLA-E typing by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

DNA was extracted from peripheral blood of recipients using QIAamp DNA Mini Kit (Qiagen, Germany), according to the manufacturer's protocol. Detection of HLA-E polymorphism was performed using PCR-RFLP [28]. PCR reaction consisted of 25 μl, containing 100 ng DNA, 10 pmol of each primer: forward primer: 5′-GGCTGCGAGCTGGGGCCCGCC-3′, reverse primer: 5′-AGCCCTGTGGACCCTCTT-3′, 1X Go Taq buffer including 1.5 mM $MgCl_2$, 1.25 U Go Taq DNA polymerase (Promega, Madison, USA) and 0.2 mM each dNTP (Thermo Scientific, Fermentas).

The PCR cycling conditions consisted of initial denaturation at 94 °C for 5 min. This was followed by 35 cycles of 94 °C for 45 s, 61 °C for 45 s and 72 °C for 45 s then final extension step at 72 °C for 7 min. The resulted fragment by visualization on 2% gel was

Table 1
Patients' characteristics

	Entire group $n = 88$	01:03 allele $n = 54$	01:01/01:01 $n = 34$	p-Value
Age				
median (range) (y)	30 (2–48)	31 (15–45)	29 (2–48)	0.21
Sex				
Male	53 (60.2)	34 (63)	19 (55.9)	0.66
Female	35 (39.8)	20 (37)	15 (44.1)	
Disease at transplantation, n (%)				
Acute myeloid leukemia	52 (59.1)	30 (55.5)	22 (64.7)	0.55
Myelodysplastic syndrome	15 (17)	9 (16.7)	6 (17.6)	
Chronic myeloid leukemia	21 (23.9)	15 (27.8)	6 (17.6)	
Stage of disease, n (%)				
Low, intermediate	79 (89.7)	47 (87)	32 (94.1)	0.45
High	9 (10.3)	7 (13)	2 (5.9)	
Prior therapy, n (%)				
No	34 (38.6)	23 (42.6)	11 (32.4)	0.46
Yes	54 (61.4)	31 (57.4)	23 (67.6)	
Gender combination (Recipient/Donor), n (%)				
M/M	29 (33)	16 (29.7)	13 (38.2)	0.23
M/F	22 (25)	17 (31.6)	5 (14.7)	
F/F	13 (14.8)	9 (16.4)	4 (11.7)	
F/M	24 (27.2)	12 (22.3)	12 (35.4)	
Source of stem cells, n (%)				
Bone Marrow	4 (4.5)	1 (1.9)	3 (8.8)	0.31
Peripheral blood	84 (95.5)	53 (98.1)	31 (91.2)	
Conditioning regimen, n (%)				
Myeloablative				
Busulfan/Cyclophosphamide	72 (81.8)	46 (85.2)	26 (76.4)	0.45
Busulfan /Fludarabine				
Reduced intensity	16 (18.2)	8 (14.8)	8 (23.6)	
Fludarabine /Melphalan				
GVHD prophylaxis, n (%)				
Cyclosporine /Methotrexate	68 (77.2)	44 (81.5)	24 (70.6)	0.41
Cyclosporine/MMF	20 (22.8)	10 (18.5)	10 (29.4)	

Low, intermediate: AML(CR1), CML (chronic phase) and MDS; High: AML (CR2) and CML (accelerated phase).