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Occurrence of minor histocompatibility antigens' disparities in allogeneic hematopoietic stem cell transplantation recipients and their HLA-matched siblings

Występowanie niezgodności słabych antygenów zgodności tkankowej w allogenicznych przeszczepieniach komórek krwiotwórczych od zgodnego w układzie HLA rodzeństwa

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Summary

We have determined the alleles of eleven minor histocompatibility antigens (mHAgs) and investigated the occurrence of immunogenic mHAgs mismatches between a donor and a recipient of allogeneic hematopoietic stem cell transplantation (alloHSCT) from HLA-matched sibling donors in 35 recipients after myeloablative conditioning between 2000 and 2008. Mismatches were either graft-versus-host or host-versus-graft directed. The frequency analysis of mHAg alleles, genotypes and phenotypes accompanied by appropriate restriction HLA antigens allowed for estimation of the probability of immunogenic mismatches. The investigation of the association of detected immunogenic mHAgs mismatches between a donor and a recipient with a course of alloHSCT is warranted.

Key words: minor histocompatibility antigens, allogeneic hematopoietic stem cell transplantation, HLA-matched sibling

Streszczenie

Oznaczyliśmy allele jedenastu słabych antygenów zgodności tkankowej (mHAg) i zbadaliśmy występowanie ich immunogennych niezgodności pomiędzy dawcą i biorcą w 35 allogenicznych przeszczepieniach komórek krwiotwórczych od zgodnego w układzie HLA rodzeństwa wykonanych z zastosowaniem przygotowania mieloablacyjnego w latach 2000-2008. Niezgodności były ukierunkowane w stronę przeszczep-przeciw gospodarzowi (GVH) lub gospodarz-przeciw przeszczepowi (HVG). Analiza częstości występowania alleli, genotypów i fenotypów, uwzględniająca występowanie odpowiednich antygenów restrykcyjnych HLA pozwoliła na oszacowanie prawdopodobieństwa wystąpienia immunogennej niezgodności. Następnym etapem pracy będzie zbadanie związku pomiędzy wykrytymi niezgodnościami mHAg pomiędzy dawcą i biorcą a przebiegiem klinicznym procedury przeszczepowej.

Słowa kluczowe: słabe antygeny zgodności tkankowej, allogeniczne przeszczepienie komórek krwiotwórczych, układ HLA, rodzeństwo

INTRODUCTION

The allogeneic hematopoietic stem cell transplantation (alloHSCT) constitutes a recommended therapy of many proliferative, especially hemato-oncologic diseases. Despite the fact, that hematopoietic stem cell transplantology develops very dynamically, and almost 40 years have passed since the first alloHSCT, early and late complications of post-transplant care remain unresolved. Early complications include conditioning toxicity (nausea, vomitus, alopecia, hemorrhagic cystitis, sinusoidal obstruction syndrome, interstitial pneumonia, thrombotic microangiopathy), pancytopenia with related infections and acute graft-versus-host disease (a-GVHD). Late complications include those related to conditioning toxicity (infertility, cataract, hypothyreosis, secondary malignancies) and chronic graft-versus-host disease (cGVHD).

Although the prognosis after alloHSCT depends mainly of the disease, long survival is being estimated in the range of 40-70%. Infectious complications and

GVHD (30-40%), organ toxicity of chemotherapy (20%) and relapse (20-30%) are the most frequent causes of failures.

The possession of a HLA-matched donor is a key requirement for alloHSCT therapy. Tissue histocompatibility is determined by genes of major histocompatibility complex (MHC), which in man is known as a HLA (human leukocyte antigens). The genes encoding HLA antigens system are located in the short arm of chromosome 6. The products of the HLA genes can be divided into class I (HLA-A,-B,-C) and class II (HLA-DP,-DQ,-DR) molecules. Class I HLA antigens are expressed on most of nucleated cells, excluding red blood cells and cells of the nervous system, while class II HLA molecules occur mainly on B cells, macrophages, dendritic cells and thymic epithelial cells. Molecules of both classes differ in structure, tissue distribution and characteristics of peptide presentation to T-lymphocytes which plays a major role in creating immunity. HLA typing- key element of donor-recipient pair matching- is managed with use of serological and more accurate bio-molecular methods based on identification of HLA-antigens encoding DNA.

The DNA typing methods include:

a) specific sequences of DNA nucleotides (SSOP – sequence-specific oligonucleotide probe),
b) DNA sequence-specific primers (SSP – sequence specific primers),
c) direct nucleotide sequencing (SBT – sequence based typing),
d) other methods such as using a hetero-duplex analysis.

Matching of HLA compatible donor is the most important single factor determining the outcome of allogeneic transplantation, affecting the possible loss of graft, the incidence and severity of GVHD and survival.

Siblings are the first to be tested in order to find an optimal donor of hematopoietic cells. The odds ratio for HLA compatibility in siblings is 1:4. The probability of having a compatible donor among siblings by a particular patient is determined by the formula $1- (0.75)^n$, where n is the number of possessed siblings. In case of the absence of siblings or lack of compliance, search of an unrelated donor is performed. When not successful, it is followed by an alternative donor search, i.e. an unrelated HLA mismatched, or donor from extended family.

The probability of finding an unrelated donor is dependent upon the prevalence of certain haplotypes in the general population. Odds ratio of finding an unrelated donor is about 1:10 000, but in case of a search of world registers which contain search determinants currently of more than 15 million donors, it is possible to find one for the majority of patients in need.

Unfortunately, failure of treatment is observed in some patients despite full HLA-match of donor-recipient pair, a state of disease remission before transplantation and the best course of transplant procedure. Excluding the possibility of incorrect HLA typing it can be suspected, that mismatched minor histocompatibility antigens (mHAgs) may be responsible. These antigens belong to a very heterogeneous group of peptides, usually composed of 9-12 amino-acids. Disparities in the mHAgs result from polymorphism of amino-acids which they are composed of, as a consequence of polymorphisms of genes encoding them. The product of each polymorphic gene in combination with molecules of the major histocompatibility complex MHC may induce a response and act as a transplant mHAg. mHAg are encoded by autosomal genes or gender genes located on the Y chromosome, which thus do not occur in women. Most of mHAgs are encoded by one immunogenic and one non-immunogenic allele, and in fact one allele determines the potential strength of their immunogenicity. mHAgs are being presented after binding to the appropriate binding site of the HLA class I or class II molecule. The dependence of mHAgs immunogenicity from the presence of specific HLA molecule possessing an adequate peptide binding site specific for each particular mHAg is called MHC restriction. Autosomal and Y-chromosome encoded mHAgs are presented in Tables 1 and 2, respectively.

The tissue distribution of mHAg varies, resulting in the diversity of the clinical reaction occurring between T-lymphocytes and mHAgs .Two of autosomal encoded mHAgs (HA-3 and HA-8) and most mHAgs encoded by the Y chromosome are present in most tissues, including skin, intestine and liver, key organs for the development of GVHD. The presence of another mHAgs is limited to selected tissues. For example: HA-1 and HA-2 are present only on hematopoietic cells. There are 11 autosomal encoded mHAgs and 2 associated with the Y chromosome described, whose expression is restricted only to hematopoietic cells. There is also a third type of mHAg tissue expression- limited to solid tumor cells.

The role of mHAgs in transplantation is diverse and is being still intensively investigated. The significant role of mHAgs has been observed in transplant rejection (HVG – host versus graft reaction). Worse survival in female recipients for which the donors were men is an example of this complication. The expression of donor Y-chromosome encoded mHAgs occurring in women following the transplantation became an aim of attack of recipient T-cells. Previous analysis also showed that disparities in the Y chromosome encoded mHAgs in the GVH direction in men who had unrelated female donor decreased the relapse rate and tended to improve the disease-free survival, but also increased the incidence of cGVHD. It was also shown that mismatches of mHAg in HVG direction had a significant impact on the higher relapse rate during the first year after alloHSCT. Many studies have explored the role of mHAg mismatches in the development of GVHD. The presence of T-cells specific for recipient mHAgs in patients presenting symptoms of GvHD has been confirmed. The discrepancy between the donor and recipient in mHAgs present on hematopoietic cells, including HA-1, HA-2 and HA-8,

Table 1. mHAg autosomal encoded.

mHAg	Restriction	Identification	Clinical trials	Protein	Tissue distribution	
HA-1	HLA-A*02	Den Haan 1998	Goulmy 1996 Tseng 1999 Gallardo 2001	HA-1	Restricted	Hematopoietic cells Bronchial Carcinomas Cervix Carcinoma Breast Carcinoma Prostate Carcinoma
HA-1/B60	HLA-B*60	Mommaas 2002	–	HA-1	Restricted	Hematopoietic cells
HA-2	HLA-A*02	Den Haan 1995	Goulmy 1996	Myosin 1G	Restricted	Hematopoietic cells
HA-3	HLA-A*01	Spierings 2003	Goulmy 1996	Lymphoid blast crisis oncogene	Broad	Hematopoietic cells Keratinocytes Fibroblasts PTECs HUVECs Melanocytes
HA-8	HLA-A*02	Brickner 2001	Akatsuka 2003 Perez-Garcia 2005	KIAA0020	Broad	Hematopoietic cells Fibroblasts
HB-1$^{H/Y}$	HLA-B*44	Dolstra 1999	–	unknown	Restricted	B cell ALL, EBV-BLCLs
ACC-1	HLA-A*24	Akatsuka 2003	Nishida 2004	BCL2A1	Restricted	Hematopoietic cells
ACC-2	HLA-B*44	Akatsuka 2003	–	BCL2A1	Restricted	Hematopoietic cells
SP110 (HwA-9)	HLA-A*03	Warren 2006	–	SP110 intranuclear protein	Restricted	Hematopietic cells IFN- gamma inducible
PANE1 (HwA-10)	HLA-A*03	Brickner 2006	–	PANE1	Restricted	Lymphoid cells
UGT2B17/A29	HLA-A*29	Murata 2003	–	UGT2B17	Restricted	Dendritic cells, B- cells, EBV-BLCLs
UGT2B17/B44	HLA-B*44	Terrakura 2007		UGT2B17	Restricted	Dendritic cells, B- cells, EBV-BLCLs
LRH-1	HLA-B*07	de Rijke 2005	–	P2X5	Restricted	T cells, B cells, NK cells, PHA blasts, EBV-BLCLs, AML
LB-ECGF-1H	HLA-B*07	Slager 2006	–	ECGF-1	Restricted	Hematopoietic cells
CTSH/A31	HLA-A*31	Torikai 2006	–	Cathepsin H	Restricted	EBV-BLCLs, AML
CTSH/A33	HLA-A*33	Torikai 2006	–	Cathepsin H	Restricted	EBV-BLCLs, AML
LB-ADIR-1F	HLA-A*02	van Bergen 2007	–	TOR3A	Restricted	–
ACC-6	HLA-B*44	Kawase 2007	–	HMSD	Restricted	–

corresponds to the degree of GVHD severity. There are also clinical data showing that donor T-cells specific for mHAg present only on recipient hematopoietic cells are critical for the maintenance of remission after transplantation, because they are responsible for graft-versus-leukemia (GVL) effect. The above observation has initiated attempts to use mHAgs in immunotherapy of proliferative diseases of the hematopoietic system. Inventing of vaccines using mHAgs peptides would be the best solution for the improvement of clinical practice. These vaccines would be used to donors and recipients in order to increase the GVL reactivity.

AIM OF THE STUDY:

- To investigate whether mHAg disparities occur in HLA matched siblings (inheriting the same HLA haplotypes),
- To investigate the impact of immunogenic mHAg disparities on the results of transplants from the related donors.

MATERIAL

The study included 68 donor-recipient sibling pairs in whom the procedure of related allogeneic hematopoietic cells transplantation has been performed in the Department of Hematology and Bone Marrow Transplantation. Medical University of Silesia, in Katowice in years 2000-2008. Three enrolled donor-recipient pairs were identical monozygotic twins.

A preliminary analysis included 35 pairs in which the recipients were 23 women and 12 men, median age of recipient was 41.7 years (range: 19-58). Detailed demographic data on the study group donor-recipient, the indications for transplant and the type of conditioning therapy are shown in table No. 3.

METHODOLOGY

DNA has been isolated from peripheral blood of related donor-recipient pairs in the Immunogenetics and the HLA Laboratory of the Regional Blood Center or in the Bio-molecular Laboratory of the Department of

Table 2. mHAg encoded by the Y chromosome.

mHAg	Restriction	Identification	Clinical trials	Protein	Tissue distribution	
A1/HY	HLA-A*01	Pierce 1999	–	USP9Y	Broad	Hematopoietic cells, fibroblasts
A2/HY	HLA-A*02	Meadows 1997	Goulmy 1996	SMCY	Broad	Hematopoietic cells, fibroblasts
A33/HY	HLA-A*33	Torikai 2004	–	TMSB4Y	Broad	Hematopoietic cells
B7/HY	HLA-B*07	Wang 1995	–	SMCY	Broad	Hematopoietic cells
B8/HY	HLA-B*08	Warren 2000	–	UTY	Restricted	Hematopoietic cells
B52/HY	HLA-B*52	Ivanov 2005	–	RPS4Y1	Restricted	Leukocytes, PHA blasts, EBV-BLCLs, B cells, Breast carcinoma, Hepatocellular carcinoma, Colon adenocarcinoma, AML, ALL Multiple myeloma
B60/HY	HLA-B*60	Vogt 2000	–	UTY	Broad	Hematopoietic cells, fibroblasts
DRB1*1501/HY	HLA-DRB1*15	Zorn 2004	–	DDX3Y (DBY)	Broad	Hematopoietic cells, fibroblasts
DRB3*0301/HY	HLA-DRB3*0301	Spierings 2003	–	RPS4Y1	Broad	Hematopoietic cells, fibroblasts
DQ5/HY	HLA-DQB1*05	Vogt 2002	–	DDX3Y (DBY)	Broad	Hematopoietic cells, fibroblasts

Abbreviations: HUVE – human umbilical vein epithelium, PTE – proximal tubular epithelium, EBV-BLCL – Epstein Barr virus transformed B – lymphoblastoid cell lines, PHA – phytohaemagglutynin
Data in table 1 and 2 are based on materials presented during „Minor histocompatibility workshop" 2005, Leiden Univeristy Medical Center; Eric Spierings: Minor H antigens: targets for tumour therapy – lecture at the conference, „Immunogenetics in haematology and stem cell transplantation", Wrocław 09.02.2006; and Spierings E., Goulmy E.: Expanding the immunotherapeutic potential of minor histocompatibility antigens. J Clin Invest 2005, 115, 3397-3400.

Hematology and BMT, Medical University of Silesia. Alleles of 11 autosomal and HY encoded mHAgs were analyzed for each donor- recipient pair with use of Dynal AllSet mHAg Typing Kit and PCR-SSP method. The study was conducted in accordance to the methodology recommended by a team of University Medical Center in Leiden (LUMC) in the Netherlands (the inventor of the method) (1). Products obtained by PCR-SSP reaction were analyzed on agarose gel, and each detected allele encoding mHAg was recorded with a letter code symbolizing an amino acid, a product of the expression of a specific region of the nucleotide. The obtained letter code of alleles encoding mHAg of related donor-recipient pairs were then incorporated online into the database db Minor LUMC (on the subpage Immunogenicity in typed Donor/Recipient Pairs), resulting in the summary of the number and direction of mHAg mismatches and type of their tissue distribution (Direction of mHAg mismatches: Recipient versus Donor [HVG], Donor versus Recipient [GVH]).

The study has been approved by the Bioethics Committee of Medical University of Silesia in Katowice on 19 May 2009.

RESULTS

The prevalence of of 11mHAg alleles and genotypes in 35 donor – recipient pairs is presented in tables 4 and 5, respectively.

Immunogenic disparities of HA-1, HA-8, HB1-H, HB1-Y, HwA-9, UGT2B17, ACC-1, ACC-2 and HY were revealed in 24 (68.6%) out of 35 analyzed sibling pairs. No mHAgs disparities were detected in remaining 11 pairs (31.4%).

The differences in mHAgs have been additionally observed in 2 out of 3 monozygotic twins.

In 16 pairs (45.7%) immunogenic disparities were HVG-directed, in 12 pairs (34.3%) GVH-directed. Bi-directional disparities have been observed in 4 pairs (11.4%).

Acute graft versus host disease has occured in 15 patients (42.8%). Chronic graft versus host disease has occured in 14 patients (40%), in 5 of them (14.2%) cGVHD was extensive. 9 patients (25%) relapsed after alloHSCT, fatal course has been observed in 8 patients (22%).

The analysis of potential influence of mHAgs disparities on alloHSCT complications is warranted.

DISCUSSION

mHAg frequency has been studied worldwide (2), in Caucasians (3) and in polish patients (4, 5). GVH-directed immunogenic disparities of mHAgs, especially of sex-related antigen HY, may be responsible for more frequent occurrence of chronic GVHD (6, 7). Graft failure also may be contributed to disparate mHAgs (8, 9). mHAg mismatch may be re-

Table 3. Demographic data.

Patient Characteristics (n-number of pts = 35)			Mean (range)	Quartiles
Age (years)	Recipient		41.7 (19-58)	32.2-47.9
	Donor		43.0 (14-60)	28.5-51.0
Time from diagnosis to allo-HCT (months)			7.2 (3-54)	6.2-10.8
			n	**%**
Sex	Donor	Male	19	54.3
		Female	16	45.7
	Recipient	Male	12	34.3
		Female	23	65.7
	Donor/Recipient	Male/Male	7	20
		Female/Female	11	31.4
		Male/Female	12	34.3
		Female/Male	5	14.3
Compatibility of major blood group	Compatibility		20	57.1
	Minor incompatibility		3	8.6
	Major incompatibility		9	25.7
	Minor and major incompatibility		3	8.6
Rh compatibility	Compatibility		26	74.3
	Donor – Recipient +		4	11.4
	Donor + Recipient –		5	14.3
Diagnosis			**n**	**%**
ALL	CR1		2	5.7
	CR > 1		1	2.8
AML	CR1		30	85.8
	CR > 1		2	5.7
Regimen			**n**	**%**
TBI + Cyclophosphamide			3	8.6
Chemotherapy	Busulfan + Cyclophosphamide		18	51.3
	Treosulfan + Fludarabine		11	31.4
	Busulfan + Fludarabine		1	2.9
	Treosulfan + Cyclophosphamide		1	2.9
	Busulfan + Cyclophosphamide + AraC		1	2.9
Source of cells	Bone Marrow		22	62.8
	Peripheral Blood		7	20
	Bone Marrow and Peripheral Blood		6	17.2

Table 4. The prevalence of of 11mHAg alleles in 35 donor – recipient pairs.

Antigen	Allele	Recipient (n = 35)	Donor (n = 35)
HA-1	H	40.0%	44.2%
	R	60.0%	55.8%
HA-2	V	72.9%	66.0%
	M	27.1%	34.0%
HA-3	T	62.7%	66.7%
	M	37.3%	33.3%
HA-8	R	43.8%	45.8%
	P	56.3%	54.2%
HB-1	H	57.1%	60.0%
	Y	42.9%	40.0%
ACC-1	Y	31.3%	23.9%
	C	68.7%	76.1%
ACC-2	D	29.2%	25.5%
	G	70.8%	74.5%
SP110 (HwA9)	R	55.4%	52.6%
	G	44.6%	47.4%
PANE1 (HwA10)	R	62.7%	62.3%
	*	37.3%	37.7%
UGT2B17	+	82.9%	94.3%
	–	17.1%	5.7%
HY	+	37.1%	51.4%
	–	62.9%	48.6%

Table 5. The prevalence of 11mHAg genotypes in 35 donor- recipient pairs.

Antigens	Genotype	Recipient (n = 35)	Donor (n = 35)
HA-1	HH	5.7%	17.1%
	HR	57.2%	48.6%
	RR	37.1%	34.3%
HA-2	VV	62.9%	51.4%
	VM	37.1%	42.9%
	MM	0.0%	5.7%
HA-3	TT	45.7%	54.3%
	TM	45.7%	37.1%
	MM	8.6%	8.6%
HA-8	RR	22.9%	25.8%
	RP	37.1%	37.1%
	PP	40.0%	37.1%
HB-1	HH	34.3%	37.1%
	HY	57.1%	57.1%
	YY	8.6%	5.8%
ACC-1	YY	5.7%	0.0%
	YC	37.1%	31.4%
	CC	57.1%	68.6%
ACC-2	DD	2.9%	0.0%
	DG	37.1%	34.3%
	GG	60.0%	65.7%
SP110 (HwA9)	RR	28.6%	22.9%
	RG	60.0%	62.9%
	GG	11.4%	14.2%
PANE1 (HwA10)	RR	45.7%	42.9%
	R*	45.7%	51.4%
	**	8.6%	5.7%
UGT2B17	++ or +–	94.3%	82.9%
	– –	5.7%	17.1%
HY	++ or +–	51.4%	37.1%
	– –	48.6%	62.9%

sponsible for GVL reaction and thus may decrease the relapse rate (10, 11).

The multidirectional influence of mHAg mismatches justifies studies aiming to determine their occurrence.

CONCLUSIONS

Immunogenic mHAg disparities occur in HLA-matched siblings inheriting same HLA haplotypes and thus they may influence the transplant outcomes. Differences in mHAg have been observed also in monozygotic twins.

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otrzymano/received: 04.05.2011
zaakceptowano/accepted: 09.06.2011

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