ORIGINAL ARTICLE

HLA-DPB1 Gene Analysis in Haematopoietic Stem Cell Transplantation

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ABSTRACT

Background: The HLA-DPB1 gene is probably one of HLA class II. genes affecting the haematopoietic transplantation. The aim of this study was to analyse the HLA-DPB1 gene and its match/mismatch in patient transplantation from unrelated HSC donor. The PCR-SSP method was used for the typing of the HLA system.

Methods and Results: The cohort consisted of 201 pairs of patients/unrelated HSC donors. Match in HLA-A, -B, -Cw, -DRB1 and -DQB1 (e.g. 10/10 match) was detected in 81 pairs. The HLA-DPB1 was tested in them. 18 different HLA-DPB1 alleles were identified in this cohort. Complete match (e.g.12/12) was detected only in 3 % of the 201 analysed pairs.

Conclusion: The probability of finding 12/12 matching unrelated HSC donors is limited due to the high percentage of mismatches and inaccessibility of previous HLA-DPB1 results.

Key words: hematopoietic stem cell transplantation, major histocompability system, HLA class II genotyping, locus, polymorphism, haplotype.

Čas. Lék. čes, 2006, 145, pp. 126–129.

INTRODUCTION

HLA genes (Human Leukocyte Antigens) the genes of the major histocompatibility complex in man are located on the short arm of chromosome 6 (6.p21), and this system occupies approximately one thousandth of the human genome. The HLA system is the most polymorphous part of the human genome. More than 2000 alleles have been described up to now (1). The HLA system follows a pattern of "autosomal dominant" inheritance. HLA genes are predominantly inherited as a complex because of its location in one part of genome. This means that every individual inherits one haplotype from each parent. Recombinations are described in approximately 1 % of cases.

Match in HLA systems between patient and donor plays a key role in successful haematopoietic stem cell transplantation (HSCT). Match in HLA class I genes in HLA-A, -B loci and in HLA class II genes in HLA-DRB locus is generally considered the most important. HLA-Cw (class I) and HLA-DQB1 (class II) genes are also considered important. There could theoretically occur approximately $29x10^9$ reciprocal combinations in these 5 loci because of the number of known alleles. But presence of individual alleles and also haplotypes differs in different subpopulations (2, 3).

The HLA-DPB1 gene is probably one of HLA class II genes affecting the HSCT (4). In recent studies where cohorts of patients were tested at the allele level the effect of HLA-DPB1 mismatch was demonstrated GvHD (graft versus host disease) and survival after transplantation (5, 6, 7). The most recent study (8) describes the influence of HLA-DPB1 mismatch on the increase risk of GvHD, but at the same time on the decrease of risk of haematological disease relapse in T-depleted grafts from unrelated donors.

Linkage disequilibrium exists between individual alleles of HLA class I and II loci. It is especially strong between HLA-DRB1 and HLA-DQB1 loci. This linkage was not detected between HLA-DRB1/-DQB1 and HLA-DPB1. There are three recombinant places between HLA–DQB1 and HLA–DPB1. These are between HLA-DNA and RING3 genes, DQB3 and DQB1 genes and inside TAP2 (9). Retrospective studies of pairs patient/unrelated donor (originally declared as matching in HLA class II) describes mismatch in HLA-DPB1 locus in 75 % of cases (6, 7, 8).

CHARACTERISTICS OF HLA-DPB1 GENE

HLA-DP is a typical HLA class II glycoprotein, composed of alpha chains (coded by HLA-DPA1 gene) and beta chains (coded by HLA-DPB1 gene). 119 alleles of HLA-DPB1 coding for 106 different protein chains and 23 alleles of HLA-DPA1 coding for 14 different protein chains are known today (1). The influence of HLA-DPA1 incompatibility on the course of post-transplantation period has not been proven. Alleles of this locus are in strong genetic linkage with HLA-DPB1. If mismatch in HLA-DPA1 is detected, mismatch in HLA-DPB1 is also detected in most cases (6, 10, 11). Therefore only more polymorphous HLA-DPB1 gene was chosen for testing.

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AIM OF THE STUDY

The aim of our study was to establish the frequency HLA-DPB1 gene alleles in Czech patients that were transplanted with haematopoietic stem cells from unrelated donors. At the same time we studied the frequency HLA-DPB1 alleles in unrelated HSC donors and mutual match in this locus between donor and patient. Our effort was to determine whether it is realistic to look for donors for patients indicated for unrelated transplantation of haematopoietic stem cells matched not only in HLA-A, -B, -Cw, -DRB1 and -DQB1 - as has been the case to date - but also in alleles of HLA-DPB1 locus.

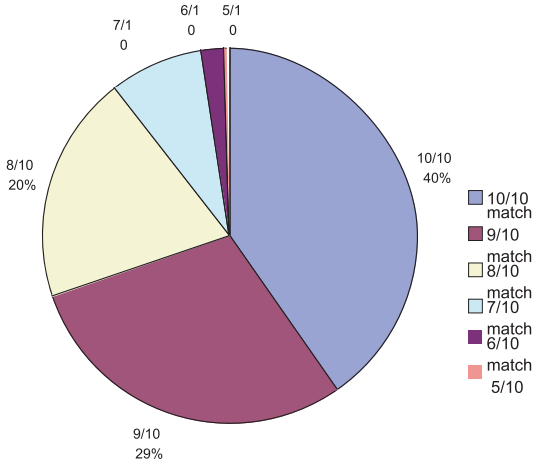

Figure 1: Division of cohort according to the grade of match in HLA-A, -B, -Cw, -DRB1 and -DQB1 loci

METHODS

DNA isolation from peripheral blood of patients and unrelated donors was performed using modified method according to Miller (12).

Kits by GenoVision based on the polymerase chain reaction using sequence specific primers (PCR-SSP) assay were used for the typing of the HLA system. Evaluation was performed using SCORE™ software, which is regularly updated in relationship with international database of HLA alleles sequences (IMGT/HLA database) (1).

For specification of PCR-SSR results in HLA-A, -B and –Cw loci, an assay of direct sequencing base typing (SBT) with commercially manufactured kits by Abbott on sequencer ABI 310 a MEGABACE™ was used. Electrophoregrams and alleles identifications were evaluated using MatchMaker™ and Assign™ software.

Tested cohort

The cohort consisted of 201 pairs of patients and unrelated HSC donors. 123 of them were adult patients transplanted in IHBT, and 78 were child patients transplanted in DPHO Motol.

Typing at the level of alleles (high, possibly medium resolution) in HLA-A, -B, -Cw, -DRB1 and -DQB1 loci was performed in the whole

Tab. 1. Results of testing HLA-DPB-1 locus

Allele	total number (patients/donors)	of that homozygotes (patients/donors)	incidence (patients/donors)**
***0101**	**27** (14/13)	**1** (0/1)	**8,6%**
0101 MR	**1** (0/1)	0	
***0201**	**35** (17/18)	**1** (1/0)	**12,0%**
0201 MR	**4** (2/2)	**0**	(11,7 / 12,3)
***0301**	**21** (10/11)	**0**	**9,0%**
0301 MR	**8** ((3/5)	**0**	
***0401**	**102** (51/51)	**20** (10/10)	**36,4%**
0401 MR	**16** (7/9)	**1** (0/1)	(35,8 / 37,0)
***0402**	**42** (23/19)	**5** (3/2)	**16,4%**
0402 MR	**11** (4/7)	**2** (1/1)	(16,7 / 16,0)
***0501**	**7** (4/3)	**0**	**2,5%**
0501 MR	**1** (0/1)	**0**	
***0601**	**5** (2/3)	**0**	**1,5%**
***1001**	**5** (4/1)	**0**	**1,5%**
***1101**	**8** (3/5)	**0**	**2,5%**
***1301**	**3** (1/2)	**0**	**0,9%**
***1401**	**4** (2/2)	**0**	**1,2%**
***1501**	**6** (4/2)	**1** (1/0)	**1,9%**
***1601**	**2** (2/0)	**0**	**0,6%**
***1701**	**9** (6/3)	**0**	**2,8%**
***1901**	**2** (0/2)	**0**	**0,9%**
1901 MR	**1** (1/0)	**0**	
***2301**	**1** (1/0)	**0**	**0,6%**
2301 MR	**1** (0/1)	**0**	
***7801**	**1** (1/0)	**0**	**0,3%**
***9401**	**1** (0/1)	**0**	**0,3%**
celkem	**324** (162/162)	**31** (16/15)	

* MR = Medium resolution

** Frequency of more than 10 %

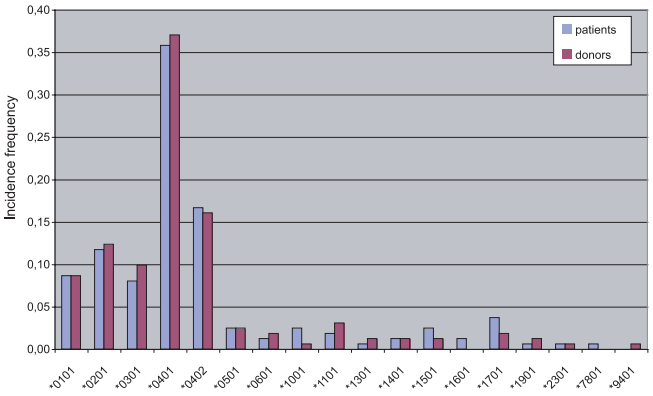

Figure 2: Frequency of incidence of HLA-DPB1 alleles in the tested cohort

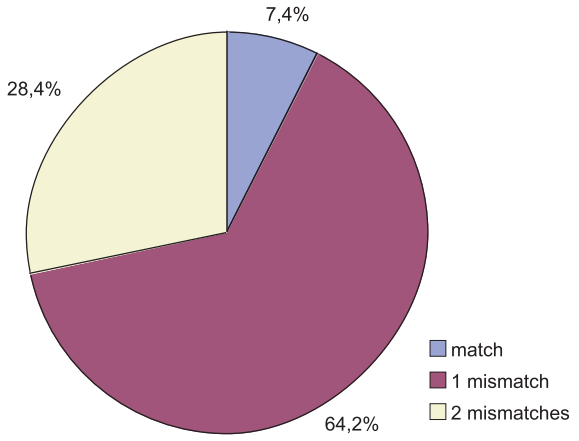

Figure 3: Division of cohort with 10/10 match according to the grade of match in HLA-DPB1 locus

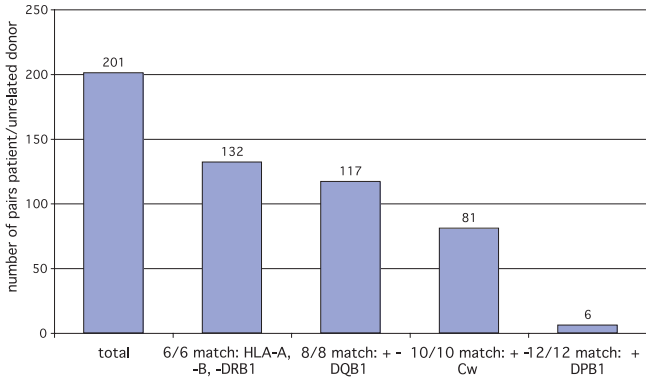

Figure 4: Division of match according to the number of tested loci

cohort within the pre-transplantation scanning for suitable donor. The degree of mutual match was determined after comparison of patients and his donor genotype. Maximal possible degree of match in both alleles of all five tested loci was determined as 10/10.

Typing of HLA-DPB1 locus using PCR-SSP (see above) assay with the same technique as for other tested loci was performed in 81 pairs with complete match in HLA-A, -B, -Cw, -DRB1 and –DQB1 (e.g. 10/10) between patient and his donor. In this part of cohort, 31 donors were from Czech registers, 38 from other European and 12 from American registers, mainly from USA. Typing of HLA-DPB1 locus was not known in tested donors in advance.

Match in HLA-A, -B, -Cw, -DRB1, -DQB1 and also in HLA-DPB1 was determined as 12/12.

RESULTS

Division of cohort according to the number of detected mismatches is shown in **Figure. 1.** Complete 10/10 match was detected in 40.9 % cases of all 201 tested pairs, i.e. in 81 pairs where HLA-DPB1 locus was subsequently tested (detailed results are shown in **Tab. 1)** In these 162 individuals HLA-DPB1 allele was definitely identified in 87.7 % cases of 324 results (two alleles in every tested individual). 28 individuals of this group were homozygotes. Results at the level of medium resolution (thus two and more possibilities) were found in the remaining 43 cases, three times in homozygote status.

Only 18 different HLA-DPB1 alleles of 119 alleles known up to now have been identified (1). **Figure. 2** shows population frequency that we have detected. It is divided into patients (black columns) and donors (white columns). The most represented allele was HLA-DPB1*0401 with population frequency more than 36 % of all tested cohort in both groups (35.8 % in patients and 37.0 % in donors - **see Tab. 1)**; of these, 21 persons (13.0 % of all 162 individuals tested) were homozygotes and 16 had medium resolution results (that is 37.2 % of 43 medium resolution results) - **see Tab**. **1.** HLA-DPB1*0201 and *0402 alleles were other identified alleles with frequency of more than 10 %. The highest incidence frequency of HLA-DPB*0201, *0401 and *0402 alleles is in agreement with the earlier published studies carried out in Czech (13,14) and Slovak (15) populations of healthy individuals. Representation of identified alleles did not significantly differ between patients and donors.

Figure. 3 shows division of cohort with 10/10 match according to the detected match/mismatch in HLA-DPB1 locus. Only 7.4 % of all tested pairs matched. More than half the pairs (64.2 %) differed in one allele, and mismatch in both alleles of this locus was detected in a further 28.4 % of all tested pairs.

HLA-A, -B and -DRB1 loci are considered to be the loci with the biggest influence on HSCT. Therefore the most available typing of donors in the international registers types in these loci, and donors are also selected according to these loci. **Figure. 4** gives division of cohort according to the match detected in particular loci and shows continually decreasing percentage of match when another tested locus is added. Different decrease in the number of detected matching donors for different HLA loci is caused by variously strong linkage disequilibrium of these loci.

DISCUSSION

Results from the study indicate that the effort to maximize HLA match between patient and his HSC donor significantly limits the probability of finding suitable donor. This limitation is especially substantial in HLA-DPB1 due to weak linkage disequilibrium between HLA-DPB1 and other HLA class II loci. (Complete 12/12 match was detected only in 6 pairs, i.e. in 3 % of all 201 tested pairs. It is not pragmatic to call for complete match. It is necessary to change the approach towards finding of tolerable mismatches. Defining of permissive mismatches has not been successfully solved yet because of its connection with considerable difficulties. Polymorphism of HLA and thus high mismatches heterogeneity between patient and donor is a big problem. It is necessary to study evaluation of the effect of particular mismatch on the background of complete match in other loci. Complex evaluation must be done with respect to non-HLA influences on GvHD and mortality-morbidity (preparative regime, CMV serology of donor, age of the donor, sex of the donor, number of pregnancies of donor, GvHD prevention, type of graft).

The international IHWG study, which collects data from all

world workplaces to NCBI (National Center for Biotechnology Information) database within the frame of Hematopoietic Cell Transplantation project, should contribute to the clarification of problem of tolerable mismatches in HLA system (16).

CONCLUSION

The effort to find 12/12 matching donors would significantly limit the probability of finding suitable donors because of the high percentage of mismatches in HLA-DPB1 locus and low accessibility of previous results of HLA-DPB1 typing. Before the effect of particular mismatches in HLA-DPB1 are evaluated, this requirement could be asserted only if previous data about typing in this locus are available.

List of used abbrevations

GvHD – Graft vesus host disease
HLA – Human leukocyte antigens
HSC – Hematopoietic stem cells
HSCT – Hematopoietic stem cell transplantation
PCR-SSR – Polymerase chain reaction
SBT – Sequencing-based typing

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This work was supported by grant No. NH/7984-3 from the Grant Agency of the Ministry of Health of the Czech Republic.

Translation: R. Mikyšková