ORIGINAL ARTICLE

The Importance of Molecular Genetic Confirmation Tests of the HLA System in Unrelated Donors of Hematopoietic Stem Cells

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ABSTRACT

Haematopoietic stem cell transplantation is a standard currative therapy for some acquired haematological diseases and inherited metabolical and immunological disorders. HLA compatibility in five loci (HLA class I -A, -B and -C and HLA class II -DRB1 and -DQB1) of the donor/recipient pair is a prerequisite for the success of haematopoetic stem cell transplantation, which represents a process of adoption donor's immunity. HLA is the most polymorphic system in the human genome, and this polymorphism can only be detected precisely by molecular genetics methods on DNA level. In the period from 2001 to 2004 we performed confirmatory testing of 366 unrelated haematopoetic stem cells donors from Czech and foreign registers for 256 patients. Only 16 % of the donors completely matched the patients in all HLA loci. We detected HLA mismatches in the samples of 81% patient/donor pairs, but these results were consonant with previous results from registers. 3 % of confirmatory samples revealed discrepancies with previous registry data. Despite of increasing number of available unrelated haematopoetic stem cell donors and the quality of registry HLA typing, the possibility of finding a completely matched donor is still limited.
Key words: HSCT- Hematopoetic Stem Cells Transplantation – HLA – Human Leukocyte Antigens – polymorphism genotyping – HLA class I – HLA class II

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HLA (Human Leukocyte Antigens) represents the main histocompatibility complex in humans. HLA is divided into several groups. HLA class I (mostly loci -A, -B, -C) are important for successful HSCT, the genes of which are expressed in all nuclear cell organisms and HLA class II (represented by a D region with transplantation important loci –DRB1, -DQB1, or perhaps DPB1), whose genes are expressed in B-lymphocytes, activated T-cells, monocytes, macrophages and dendritic cells.

Genes of the HLA system code surface glycoproteins that bind peptide fragments of proteins produced by cells (class I), digested by the cell (class II), respectively. Subsequently, they are presented on the cell surface in order to be identified by T-lymphocytes. Most of these glycoproteins are extremely variable due to high polymorphism of HLA genes. This is detectable on a DNA level. In a population, hundreds or thousands of allelic forms exist in one locus. They represent a co-dominant form of heredity and are inherited as whole haplotypes (a heterozygote inherited two different signs, alleles, a homozygote inherited one). The high level of HLA variability is protective on the level of an individual and increases the probability of a successful immune response and survival on the level of a population.

Hematopoietic stem cells are precursors of immunocompetent cells responsible for triggering an immune response. HSCT ensures adoption of the donor immune system. Incompatibility of surface antigene structures coded by HLA class I and II alleles may lead to graft rejection, HvGR (host versus graft reaction) or graft versus host reaction, GvHR, which is a serious or even fatal complication of such a procedure.

For donor-acceptor compatibility it is important to find compatibility in five loci of the HLA: HLA-A,-B,-C,-DRB1, -DQB1; in heterozygotes, there is thus an optimal compatibility 10/10 HLA alleles.

PATIENTS AND METHODS

The strategy in searching for a suitable donor

The search for a suitable donor indicated for HSCT is first started inside the family. The highest probability to find an identical HLA individual is among siblings. The probability to find an identical HLA sibling can be estimated using a formula $(1\text{-}0.75)^n$, where n represents a number of siblings. In biological parents only HLA haploidentity can be sure. In case of a detection of a rare trait, this search is initiated inside the extended family, inside a branch carrying this trait. If we do not find a suitable donor among relatives, the search is continued in the registers of volunteer bone marrow donors (MUD), first in Czech registers in Prague (1) and Pilsen (2) and further on in international registers (the search is organized in national registers via an EMDIS network) (3).

Search and assessment of HLA compatibility in potential unrelated HSC donors

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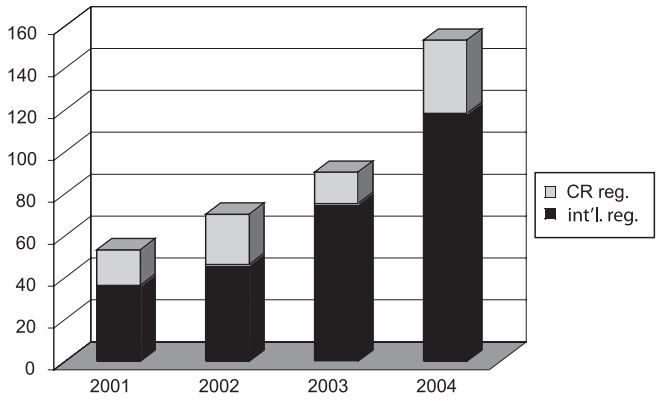
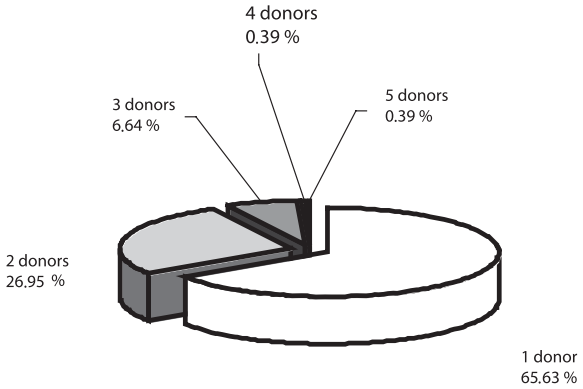
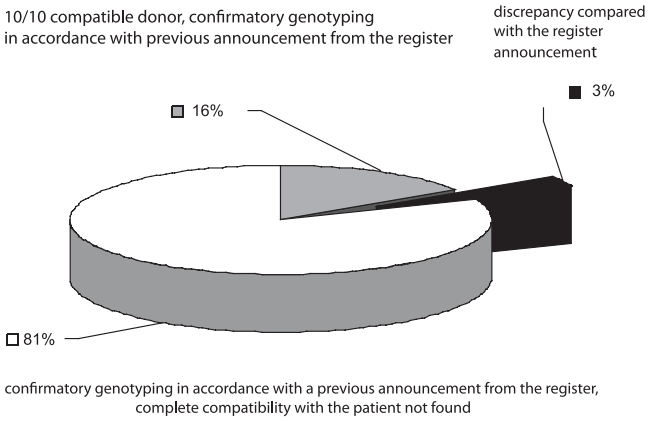


Graph 1: Number of standardised unrelated donors at the IHBT



Graph 2: Division of patients according to the number of standardised donors



Graph 3: Compatibility between a patient and a selected donor

HLA genotyping performs HLA compatibility assessment in patients and their unrelated donors for adult patients of the Institute of Hematology and Blood Transfusion and children of the Clinic of Pediatric Hematology and Oncology of the 2nd Medical Faculty and the Faculty Hospital Motol. These examinations are accredited by the Czech Institute of Accreditation (CIA) based on the CSN EN ISO/IEC 17025 and CSN EN ISO 15189.

The degree of typing of the HLA system of donors in the registers is different and its quality has changed from serotyping to genotyping. The donors are regularly typed in the HLA –A, -B loci using serology and genotyping of allelic group (low-resolution=LR, e.g. HLA-A*02), or a specific allele was determined (high-resolution=HR, e.g. HLA-A*0201). HLA-DRB1 locus is always genotyped at least on the LR level, often medium resolution (using the international writing code ambiquit, i.e. ambiguous results), but in many cases HR. The data about the HLA-C and -DQB1 alleles loci are not always known.

After seeking, claiming and sending the peripheral blood sample from unrelated donors, confirmatory HLA genotyping is performed in parallel with completing genotyping of the patients (usually the HR HLA-C locus). Confirmatory sampling from unrelated donors is performed in several steps based on the results from the register. In preference, the homozygosity on particular loci, presence of a specific allele, i.e. HR, is tested. Compatibility evaluations of the tested HLA features of the patient and his potential donor are constantly performed. The discovered differences are announced to the physician that indicates HSCT and the acceptability of detected discrepancies are consulted.

Methods of HLA genotyping

When genotyping a patient and his HSC donor, we generally use peripheral blood from which we isolate DNA using an automatic machine GenoM-6 (GenoVision). Polymerase chain reaction with sequence specific primers (PCR-SSP) with commercially manufactured kits by GenoVision is currently used for routine HLA genotyping. These reflect the still increasing knowledge about HLA polymorphism. This method performs reaction with about 150 to 270 primers, depending on the genetic situation of an individual. After amplification, electrophoretic detection of the PCR product on the agarose gel is performed, followed by photo documentation of the results. Determination of a specific result is performed using computer software SCORE TM comparing the sequences of primers in individual kits with the complete database of known HLA alleles in the IMGT/HLA database (4). Except for this procedure, a method of direct sequencing (SBT) is applied, using kits of the Abbott followed by sequence analysis using the Assign SBT TM software.

Genetic analysis of the results

The decisive part of genotyping is formally genetic analysis, i.e. general evaluation of the HLA genotyping results including family studies, if performed, evaluation of binding imbalance of allele loci HLA-DRB1 and HLA-DQB1, evaluation of binding imbalance of -DRB1 allele loci with allele loci HLA-DRB3-5, association assessment of allele HLA-B loci with Bw4 or Bw6 epitopes, respectively, correlation of a result of HLA genotyping with previous results announced from other laboratories, excluding the presence of zero alleles based on correlation with announced serotyping, assessment of nomenclature equivalents of serotyping and letter coding of ambiguous results.

RESULTS

In the years 2001-2004 we performed 366 confirmatory examinations in Czech and foreign unrelated donors (Graph 1) for 256 patients. These donors were selected based on typing of our patients and preliminary results announced from registers. Graph 2 presents division of patients based on the number of their standardized unrelated donors.

Graph 3 presents the summary of results of the correlation between a patient and a donor in the group defined above. Concerning the total of 366 potential suitable donors, only 57 (i.e. 16 per cent) HLA donors completely matched the patient; i.e. we found allelic compatibility in all tested loci. (The examples of typing of several donors for one patients, the last having a 10/10 correlation, is presented in Table 1). In 297 donors (i.e. 81 per cent) we found one or more mismatch in alleles or even allelic group between the patient and the potential donor. In this group, however, confirmatory genotyping was not in disagreement with preliminary results announced from the register. In 12 donors (i.e. 3 per cent) we found discrepancies during confirmatory testing from the results announced from the register. The specification of discrepancies is presented in Table 2.

DISCUSSION

The reasons for finding such a low number of a completely identical donor (only 16 per cent in the tested group) may be presented as follows:

Tab. 1: Example of searching for a donor with a 10/10 compatibility.

Patient p. n.

HLA-A		HLA-B		HLA-C		HLA-DRB1		HLA-DQB1	
*0101	*0205	*3501/41/42	*5001	*0401/08	*0602/09	*0701	*1101	*0202	*0301

Unrelated donors:

donor 1 sex: M

HLA-A		HLA-B		HLA-C		HLA-DRB1		HLA-DQB1	
*0101	*0205	*3503	*5001	*0401	*0602/09	*0701	*1112	*02	*0301

Note: Discrepancy in the V -DRB1 locus compared with announcement from the register.
Compatibility between a patient and this donor - 8/10.

Donor 2 sex: F

HLA-A		HLA-B		HLA-C	HLA-DRB1		HLA-DQB1	
*0101	*0205	*3502/04	*5001	*0602	*0701	*1104	*0202	*0301

Note: Compatibility between a patient and this donor - 7/10.

Donor 3 sex: M

HLA-A		HLA-B		HLA-C	HLA-DRB1		HLA-DQB1	
*0101	*0205	*3502/04	*5001	*0602	*0701	*1104	*0202	*0301

Note: Compatibility between a patient and this donor - 7/10.

Donor 4 sex: F

HLA-A	HLA-B	HLA-C		HLA-DRB1	HLA-DQB1
*0201	*3502/03/04/06/13/34	*04	*06		

Note: Typing stopped after finding several incompatibilities.

Donor 5 sex: M

HLA-A		HLA-B		HLA-C		HLA-DRB1		HLA-DQB1	
*0101	*0205	*3501/41/42	*5001	*0401/08	*0602	*0701	*1101	*0202	*0301

Note: Compatibility between a patient and this donor - 10/10.

HLA polymorphism is extreme. Currently 2088 alleles are defined (as to 31st August 2005) and their number keeps on rising (4).

Preliminary typing performed in registers of HSC donors is still not satisfactory. It mostly concerns only allelic group typing or serotyping of HLA-A, -B and -DRB1. HLA-C a -DQB1 loci typing is often omitted.

Typing in registers may be false (3 per cent of discrepancies found in our study). The reasons for these discrepancies may be divided into the following groups:

False homozygosity (five cases in HLA-B locus in our study).

False serotyping (it mostly includes confusion in similar phenotypes, in our study 3 cases).

False typing in allelic groups (two cases) or in an allele within the same allelic group (one case).

Administrative mistake or a human mistake in cases when an announcement does not correlate with the genotype of the tested sample. This is mostly a mistake in writing when dictating the results or confusion in samples for typing in the register, or during blood taking for confirmation examination (one case).

In patients for whom no completely HLA unrelated donor was found, it is necessary to consider non-HLA factors and possibly accept a donor not quite compatible in HLA. This decision-making is in the competency of the physicians responsible for performing HSCT. The study groups in particular institutions are usually small, heterogenic and therefore statistically hard to evaluate. The global information concerning a detailed HLA compatibility in transplanted patients including the post-transplantation development are concentrated in the International Histocompatibility Working

Tab. 2. Specification of discrepancies betwen confirmation examinations and results announced from the registers of unrelated donors of HSC

Announcement	Our results	Discrepancy
B*07	B*07,*56	False homozygosity
B49	B*13,*49	False homozygosity
B7	B*07,*13	False homozygosity
B*44	B*15,*44	False homozygosity
B*51	B*51,*52	False homozygosity
B70	B*1501(B62)	False serotyping
A1,25	A*01,*26	False serotyping
A3,11	A*03,*32	False serotyping
DRB1*04,*16	DRB1*04,*15	False allelic group
A*31,*68	A*30,*68	False allelic group
DRB1*1101	*1112	False allele
DRB1*12XX,*15XX	DRB1*0401	Results confusion

Group - Hematopoietic Cell Transplantation project in the NCBI dbMHC HCT database (5).

CONCLUSION

Today HSCT is a part of standard protocols in the treatment of hematological malignancies, some metabolic disorders and immunodeficiencies. The knowledge of extreme HLA polymorphism is still growing due to an increasing number of individuals examined all around the world (5) and also as a result of improved methods of detection. Concerning the fact that this increase is followed by improved data storage in registers of unrelated donors, a completely HLA-compatible donor is still available for a limited number of patients.

List of used abbreviations

CIA - Czech Institute of Accreditation
EMDIS - European Marrow Donor Information System
GvHR - Graft versus Host reaction
HLA - Human Leukocyte Antigens
HR - high-resolution – (detection on the level of alleles)
HSC - Hematopoietic Stem Cells
HSCT - Hematopoietic Stem Cells Transplantation
HvGR - Host versus Graft Reaction
IMGT - International Histocompatibility Working Group
LR - low-resolution – (detection on the level of allelic groups)
MUD - Marrow Unrelated Donor
PCR - SSP – Polymerase Chain Reaction- Sequence Specific Primers
SBT - Sequencing-Based Typing

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