ACTA MEDICA LITUANICA. 2005. VOLUME 12 No. 3. P. 32–36

Present status of HLA typing for haematopoietic stem cell transplantation in Lithuania

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Objective. The present paper summarizes development of donor search for allotransplantation of haematopoietic stem cells (HSC) in Lithuania during a period of seven years (1998–2005). This paper considers the topics of strategy and methods of histocompatibility typing and creating a legal and organizational frame of the functioning of the Lithuanian National Bone Marrow Donor Registry.

Materials and methods. Till 2003, typing for HLA antigens was performed by the standard microlymphocytotoxicity method. Low- and high-resolution DNA-based HLA typing method, as the essential condition for the creation of Lithuanian National Bone Marrow Donor Registry, has been established under the auspices of the Europdonor foundation.

Results. Primary and control typing for allogeneic HSCT of 283 patients and their families was performed from January 1, 1998 till January 1, 2005. HLA identical donor is provided in Lithuania by core family donors typing (CFDT) in 11.1–52.9% of cases. Extended family donor search (EFDS) has been initiated in 14 cases; in total, 73 potential family donors have been examined. On 8 November 2004, Lithuanian Register was officially included into the Bone Marrow Donor Worldwide (BMDW); the total number of Lithuanian donors by 24 May 2005 was 199.

Conclusions. CFDS should be carried out whenever possible. In case of unsuccessful CFDS, unrelated donor search should be the subsequent type of search. Initiation of an EFDS is acceptable if the patient's HLA-A, -B, -DRB1 phenotype is absent amongst donors in BMDW.

Key words: HLA matching, donor search, haematopoietic stem cell allotransplantation

INTRODUCTION

Haematopoietic stem cell transplantation (HSCT) has become an accepted form of therapy for haematopoietic malignancies. It is also employed for solid cancers, hereditary blood disorders, immunodeficiencies and certain metabolic disorders. This treatment modality currently is successfully applied even in elderly patients (1, 2). Nevertheless, donor–recipient identity for tissue antigens (HLA), valuable in organ transplantation, is still critically important for good clinical results in transplantation of haematopoietic stem cells, routinely called bone marrow transplantation. While in organ transplantation selection is provided in the direction "donor–recipient" (to whom this organ should be given as the "best match"), for the HSCT recipient–donor selection is basically different; it is actually best donor selection for a recipient, sometimes from a huge amount of potential donors.

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MATERIALS AND METHODS

The ideal allogeneic donor for HSCT is a genotypically identical sibling, *i.e.* one who shares with the patient both parental haplotypes, which are identical by direct descent (3, 4). In this case the donor and the recipient are matched at the genomic level for the entire Major Histocompatibility Complex (MHC), *i.e.* two-haplotype match. However, these will only provide transplants for about 30% of patients waiting for transplantation. The lack of family donors has prompted a number of groups to explore the use of volunteer unrelated donors. Unrelated donors

are generally only phenotypically matched for the HLA class I and class II alleles; this level of matching does not necessarily mean that the donor and the recipient are matched for all of the possible epitopes on the class I and class II molecules nor for the numerous other polymorphic genes within the MHC. Within families it is also possible to identify individuals (*e.g.*, parent, uncle, aunt or cousin) who share one haplotype which is identical by descent and who are phenotypically matched on the non-shared haplotype. It is important to note that the results of matched unrelated pairs transplantation and one haplotype-matched siblings clearly show that the degree of HLA mismatch is significantly related to both the graft failure and the incidence of graft-versus-host disease (GVHD) (5–7).

In Lithuania, HLA research was started in 1969, when the first tissue typing laboratory at Vilnius University Heart Surgery Clinic was established (8). Till 2003, typing for HLA antigens was performed by means of a standard lymphocytotoxic assay still applied in organ transplantation services all over the world. HLA typing by conventional serology is based on the complement-dependent microlymphocytotoxicity test and uses highly selected HLA antigen-specific alloantisera from multiparous women or monoclonal antibodies. Since the beginning of the year 2003, the HLA laboratory has started using molecular methods for low resolution typing of Class II (HLA-DRB1, -DQB1) and Class I (HLA-A, -B, -C) antigens, as well as high resolution Class II typing for confirmatory testing of donors and recipients before the transplantation. Molecular (DNA-based) HLA typing relies on the polymerase chain reaction (PCR) for amplification of particular gene segments, and in Vilnius is currently performed by PCR-SSP, utilizing sequence-specific primers (SSP) for identification of absence or presence of a particular nucleotide sequence within the amplified gene segment.

Lithuanian immunogeneticists and haematologists agreed to apply the German consensus for donor search strategy, histocompatibility loci to be typed and histocompatibility typing techniques to be used (3). At the first stage, core family search is performed, *e.g.*, HLA-A, -B, -C antigens in the patient and patient's siblings are typed using serology typing or low resolution PCR-SSP typing. If a class I identical sibling is found, he/she and the patient are typed for HLA-DR antigens. If no identical sibling is identified, unrelated donor search at the Bone Marrow Donors Worldwide (BMDW) is started. Extended family search is initiated in special cases when no unrelated donor is available. This is particularly feasible if there is a history of consanguinity in the family, homozygosity or sharing of antigens between the parents, or if the patient has one or two high frequency haplotypes, especially in the case of rare HLA antigens in the family.

RESULTS

Currently for allogeneic haematopoietic stem cells transplantations primary and control typing of patients and their families is performed at Vilnius University Hospital Santariðkiø Klinikos (VUHSK) with the constantly growing workload (from 15 families per year in 1998 to 55 families in 2004) (Fig. 1). In Lithuania, haematopoietic stem cell transplantation is performed at VUHSK for adults since 1999 (Head of the BMT Unit I. Trociukas); in 2002 a HSCT unit for children at Santariskes Children's Hospital was opened (Head A. Savinas). The latter uses the facilities for HLA typing, leukemia immunophenotyping and leucopheresis of VUHSK. As one can see in Fig. 2, while performing core family donor search (CFDS), phenotypically identical donors for adults are provided in 31.8–51.9% and for children in 11.1–50.0% of cases. We should note that in some families the sick child is the only one, diminishing the probability of a suitable family donor nearly to zero. In case of lack of core family donor, extended family donor search (EFDS) has been initiated in 14 cases; in total, 73 potential family donors have been examined. Only in one case, presented in Fig. 3, EFDS was successful, and one antigen-mismatched aunt was found.

An essential condition of the further development of HSCT in Lithuania was creation of Lithuanian National Bone Marrow Donor Registry. Work was performed under the auspices of the Europdonor Foundation as part of IST programme STEMNET (contract number IST-2000-26117), and included introduction of HLA typing using molecular biology techniques, applying quality control measures, including preparation of Standard Operation procedures, as well as establishing an information technology system for the Registry needs.

HLA typing and quality assurance is performed considering Standards for Histocompatibility Testing (9). International quality control, Proficiency testing of HLA Class I typing for Central/East Europe, organized by L. Hirszfeld Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences (Wroclaw, Poland) is in use. Standard operation procedures (SOPs) were prepared in consent with those of BMDW and World Marrow Donor Association (WMDA) (10, 11), creating a frame of the functioning of the registry for HLA typing, other laboratory investigations, personal data handling and security aspects for human cell transplantation. Before testing, free informed consent by a volunteer donor is given on the basis of information provided by a medical professional; informed consent and concurrent information are prepared in agreement with WMDA recommendations (12). The volunteer donor is also informed that any disclosure of information that could identify him/her is prohibited, and

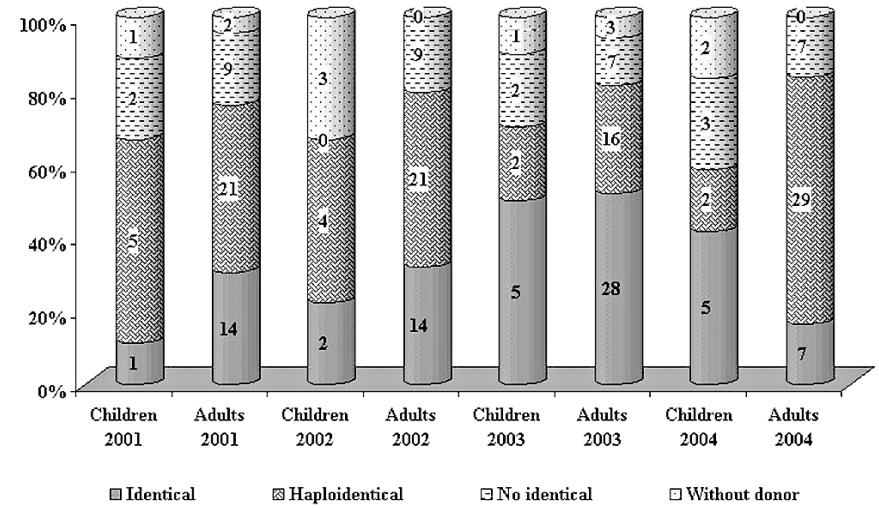

Fig. 1. Core family donor search

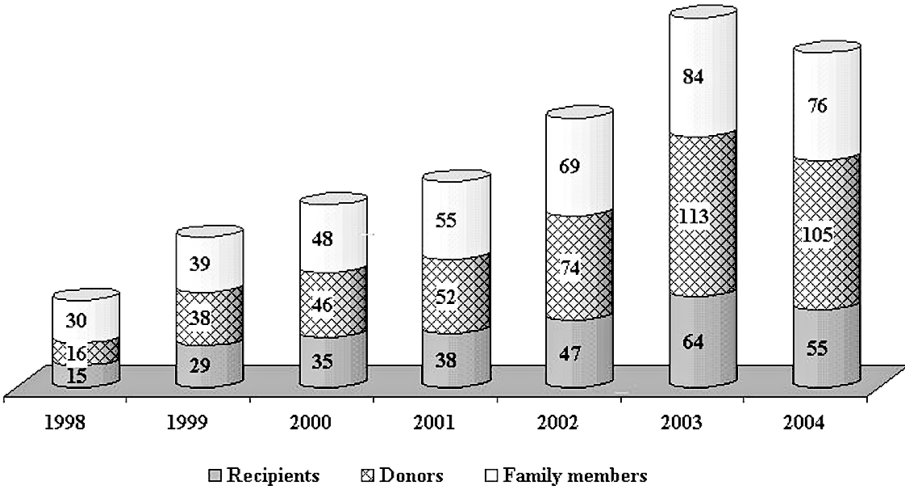

Fig. 2. HLA typing for core family donor search

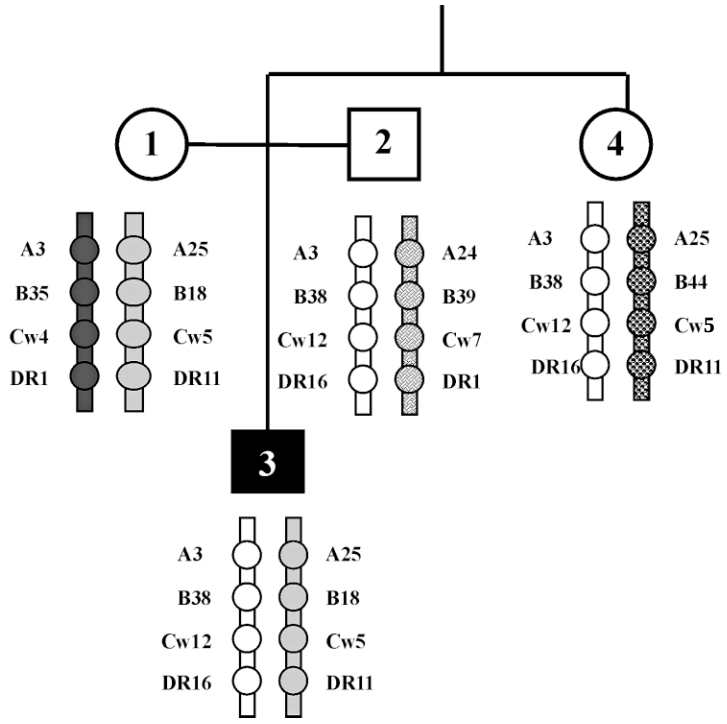

Fig. 3. An example of extended family donor search

that donor will not know the identity of a possible recipient of his/her stem cells.

The first Lithuanian unrelated donor was typed on 9 May 2003. Sixty-six donors were registered till 8 November 2004, when the Lithuanian Register was officially included into the BMDW. The workload is constantly growing; *e.g.*, from 1 January 2005 till 24 May 2005 133 potential unrelated donors were HLA-typed and registered. The total number of Lithuanian donors by 24 May 2005 was 199, 192 of them (96%) HLA-A, -B, -DR typed, 189 – DNA Class II and 40 – DNA Class I typed. Currently the participants of the BMDW are 54 stem cell donor registries from 40 countries and 38 cord blood registries from 21 countries; the up-to-date number of donors and cord blood units is 9,745,372 (last updated: 24 May 2005) (13).

DISCUSSION

Donor–recipient identity for tissue antigens (HLA) is critically important for good clinical results in allotransplantation of haematopoietic stem cells. An HLA mismatch is perhaps the strongest risk factor for outcomes after HSCT (14, 15). A major cause of morbidity and mortality following allogeneic HSCT is the development of graft-versus-host disease (GVHD) (5, 7, 14, 15, 17), caused by the T cells in the donor marrow, which recognize foreign determinants on the new host and initiate an immune response against them. This principle is well illustrated by HSCT in the treatment of children with severe combined immunodeficiency. These children accept the graft without immunosuppression and by T cell depletion of donor marrow the incidence of

GVHD is markedly reduced, even in one-haplotype mismatched sibling combinations. When GVHD did occur it was usually associated with placental transmission of maternal T cells, so the disease was actually graft-versus-graft as opposed to GVH (6). These observations pinpoint the role of T cells in GVHD. Accordingly, the incidence of GVHD in HSCT for haematological malignancies can be reduced by T cell depleting prior to infusion, but unfortunately this abrogates a beneficial effect of the T cells, that of graft-versus-leukaemia (GVL) effect. Transplantation of a matched unrelated donor results in an enhanced graft-versus-leukaemia (GVL) effect; this might be explained by the difference of HLA compatibility between HLA genotypically identical siblings *versus* unrelated donors who are matched for HLA-A, -B and -DRB1 (16), and post transplant relapse rates are lower than after HLA-matched related grafts (15). On the other hand, the basis for a higher incidence of graft failure after unrelated donor transplantation is the greater degree of genetic disparity between unrelated and related donor–recipient pairs (15).

Only very few patients are lucky enough to have an ideal syngeneic donor – identical twin. Twin transplantation is associated with a similar relapse risk and a trend for better overall survival in comparison to transplantation from an HLA-identical sibling (17). In order to give the best possible chance to the patient without ideal donor, all other donor search strategies should be in use. For Caucasian patients, the probability of identifying an HLA-matched donor through an unrelated donor search is clearly higher than through an EFDS and has meanwhile reached the percentages of > 80% *vs.* <10% (3). One step to transplantation using unrelated donors in Lithuania was creation of the Lithuanian Bone Marrow Donor Registry. Initially the Registry was sponsored by the IST programme STEMNET (contract number IST-2000-26117), by Lithuanian Society of Parents and Guardians of Children with Oncohaematological Diseases “Paguoda”, as well as by the Foundation “Help for Lithuanian Children” established under the patronage of Maestro Mstislav Rostropovich, internationally acclaimed and acknowledged as the world’s greatest living cellist. Starting from the end 2004, State Patient Fund had started to cover the expenses for the unrelated donor typing, international searches and stem cell transplantations, facilitating the finding of donors for recipients in need.

Stem cell transplantation using unrelated donors or cord blood units has become a clinical reality, but many challenges remain. The dimension of HLA polymorphism, *i.e.* the number of alleles of an HLA locus, is much higher than that presumed before a number of years. It is often difficult, or even impossible, to select a well-matched unrelated BMT donor. Although molecular typing has contributed to a more reliable HLA typing, it has also led to an enormous increase in the number of HLA alleles, ensuring constant changes of HLA typing methods, search and mismatch acceptability strategies.

CONCLUSIONS

1. Core family donor search (CFDS) should be carried out whenever possible; beside the siblings, the patient’s parents should also be tested in order to identify the maternal and paternal HLA haplotypes of the patient by means of segregation analysis. Such search is successful in about 30% of cases.

2. In case of unsuccessful CFDS, unrelated donor search should be the subsequent type of search, successful in >80% of cases.

3. Initiation of an extended family donor search is acceptable if the patient’s HLA-A, -B, -DRB1 phenotype is absent amongst donors in Bone Marrow Donors Worldwide (BMDW); in Lithuania such search was successful in 7% of cases.

Received 1 June 2005
Accepted 6 June 2005

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HLA TIPAVIMAS KRAUJODAROS KAMIENINIØ LÀSTELIØ TRANSPLANTACIJAI. LIETUVOS PATIRTIS

S a n t r a u k a

Vilniaus universiteto ligoninës Santariðkiø klinikos Klinikinës imunologijos laboratorijoje nuo 1998 m. atliekama donorø paieðka kraujodaros kamieniniø làsteliø transplantacijai (KKLT). Iki 2003 m. audiniø suderinamumo antigenai buvo nustatomi standartiniu mikrolimfocitotoksiniu metodu. Molekulinio tyrimo metodai (PGR – þemos ir aukðtos skiriamosios gebos) buvo pradëti taikyti kuriant Lietuvos nacionaliná kraujodaros kamieniniø làsteliø registrà. Iki 2005 m. sausio mën. tipuoti 283 ligoniai ir jø ðeimø nariai. Lietuviø ðeimose fenotipiðkai identiðkas donoras buvo rastas 11,1–52,9% atvejais. Ieðkant donorø artimoje giminëje buvo iðtirta 14 ligoniø ir 73 jø ðeimø nariai kaip potencialûs kraujodaros kamieniniø làsteliø donorai; vienam recipientui rastas tinkamas donoras. 2004 m. lapkrièio 8 d. Lietuvos registras buvo átrauktas á Pasauliná kaulø èiulpø registrà (BMDW – Bone Marrow Donor Worldwide), kuriame tarp 9,745,372 donorø yra 199 Lietuvos donorai (2005 geguþës 24 d.). Neradus identiðko donoro tarp artimø ar tolimesniø giminaièiø yra galimybë surasti tinkamà donorà Pasauliniame kaulø èiulpø registre.