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HLA-C and HLA-DQB1 compatibility in unrelated cord blood transplants

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ABSTRACT

Background and Objective. **Umbilical cord blood (UCB) cells have been definitively proved to be a source of hematopoietic stem cells with repopulating capacity when transplanted into pediatric hosts with neoplastic or non-neoplastic disease. Moreover, due to the immaturity of the UCB lymphoid compartment, these transplants are usually associated with a low incidence and severity of GvHD. This clinical observation and the immaturity of the UCB lymphoid compartment, justify the acceptance of UCB units which differ from their recipient by 1 or 2 HLA antigens of the six HLA A, B and DRB1 antigens conventionally typed. Whether the number and type of HLA disparities affect clinical outcome of UCB transplants has not, however, yet been clearly demonstrated.**

Design and Methods. **In the present study on 14 pediatric patients with high risk leukemia transplanted with UCB from unrelated donors, evaluation of HLA compatibility was extended to HLA-C and DQB1 genes and correlated to the engraftment rate and occurrence of GvHD. Conditioning regimen and GvHD prophylaxis were identical in all cases. HLA-A and B antigens were typed by serology, whereas DNA based methods were used to define HLA-C gene groups, and HLA-DRB1 and DQB1 alleles.**

Results. **Conventional HLA-A, B and DRB1 typing demonstrated that 12 recipient/donor pairs differed at one HLA locus, while 2 pairs had 2 HLA disparities. The extended HLA-typing showed that only one out of the six pairs with a different HLA-A locus had additional mismatches at HLA-C and DQB1 loci, whereas all the remaining 8 pairs, which already differed at HLA-B and/or DRB1 loci after conventional typing, had additional HLA-C and/or DQB1 mismatches (p = 0.002). By contrast, engraftment rate and occurrence of GvHD did not significantly correlate with level of HLA-mismatches even after extended HLA-typing.**

Interpretation and Conclusions. **The present data show that additional mismatched HLA-C and/or DQB1 antigens are significantly more frequent in pairs which after conventional HLA-typing differed at HLA-B and/or DRB1 loci, than in those showing one HLA-A mismatch. This observation provides an additional criterion for selection of UCB donors with the closest HLA-match when more than one unit are available. We did not, however, observe any correlation between engraftment rate, occurrence of GvHD and degree of HLA disparities detected either by standard or extended typing. These data support the notion that certain HLA differences do not affect the clinical outcome of UCB transplants and indicate that the expensive and time consuming molecular typing of HLA-C and DQB1 loci might be avoided for UCB donor selection.**

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Key words: umbilical cord blood, unrelated donors, HLA-C, HLA-DQB1, compatibility

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Hematopoietic stem cells in umbilical cord blood (UCB) from an HLA identical donor were used to transplant a patient with Fanconi's anemia for the first time in 1988 by Gluckman *et al.*[1] Since this first case, the number of UCB collections and transplants has increased very quickly. To date, preliminary multicenter analyses show encouraging results in hematologic patients receiving related or unrelated UCB.[2-6]

Efforts are now being made to establish common citeria for UCB banking, search and transplant procedures. In particular, as far as concerns HLA compatibilty between recipient and donor, search procedures include serologic typing of HLA-A and B antigens, and high resolution molecular typing of HLA-DRB1 genes. Due to the low number and relative immaturity of UCB lymphoid cells and to the observed low incidence and severity of GVHD after UCB transplants, it is usually accepted that UCB units might differ from the recipient host by 1 or 2 HLA antigens.

Since, however, available data are mostly based on retrospective multicenter studies in which different pre-transplant conditioning regimens were used and high resolution molecular typing for class I and class II antigens for several recipient/donor pairs is lacking,[5] relationships between UCB engraftment rate, occurrence of GVHD and HLA disparities have not yet been clearly identified.

Based on these considerations, in present study on 14 patients receiving an identical conditioning regimen and transplanted with UCB from unrelated

donors, we have extended the standard HLA-A, B and DRB1 typing to HLA-C and DQB1 genes and correlated the number of mismatched antigens to engraftment rate and occurrence of GVHD.

Design and Methods

Patients

From October 1995 to December 1997, 14 consecutive patients with high risk leukemia received an UCB transplant at the "G. Papa" Bone Marrow Transplantation Unit of the "La Sapienza" University in Rome. Table 1 summarizes the clinical characteristics and outcome of the 14 patients studied. The disease status of the patients at the time of transplantation was as follows: acute lymphoblastic leukemia (ALL) in second complete remission (CR) of disease: 7 patients; ALL in second relapse (BM blast cells < 30%): 3 patients; ALL in 1st CR: 1 patient; acute myeloid leukemia (AML) in 1st CR: 1 patient; AML in 2nd CR: 1 patient; chronic myeloid leukemia (CML) in accelerated phase: 1 patient.

DNA extraction

Cell nuclei were prepared from whole fresh or frozen EDTA bone marrow, peripheral venous blood and umbilical cord blood samples, and were lyzed by a TRITON-X-100 based method. High molecular weight DNA was extracted according to the salting-out method described by Miller *et al.*[7] after incubation in proteinase-K 10 mg/mL (Boehringer-Mannheim) at 65°C for 1 hour. DNA was then resuspended in a TRIS-EDTA solution 1 X.

HLA typing

Serologic typing for HLA-A and B antigens was determined by standard complement-dependent microcytotoxicity test.[8] Molecular typing of HLA-C (low resolution), HLA-DRB1 and HLA-DQB1 (high resolution) was performed with a sequence specific-primers polymerase chain reaction (SSP-PCR),[9] using Dynal (Oslo, Norway) commercial kits according to the manufacturer's instructions. These procedures allow definition of all HLA-C gene groups and DRB1 and DQB1 alleles recognized by the WHO Nomenclature Committee for factors of the HLA System.[10]

Unrelated donor selection

Searches for potential unrelated UCB donors were performed by the UCB Banks of Milan, New York, Dusseldorf, Paris, Barcelona, Israel. The initial matching criterion for the selection of a cord blood unit was that it should share at least 4 HLA antigens with the potential recipient. HLA compatibility was evalauted by a standard procedure which included serologic determination of HLA-A and B antigens and molecular high resolution typing of HLA-DRB1 genes. Of the 14 UCB units transplanted, 7 units were provided by the Milan Cord Blood Center, 6 units by the New York Blood Center and the remaining unit by the Heinrich Heine University of Dusseldorf, the "German UCB" bank. The median number of viable nucleated cells infused was 2.95×10^7/kg (range 1.4-7.9).

Transplant procedures

All patients were prepared with an identical conditioning regimen consisting of fractioned TBI (200 cGy

Table 1. Main clinical characteristics at time of transplant and clinical outcome of the 14 patients receiving UCB graft.

UPN	Sex/age	Weight	Disease status at transplant	No. of mismatched HLA antigens: Conv. typing (6 loci)	No. of mismatched HLA antigens: Ext.typing (10 loci)	Presence and grade of GVHD	Engraftment	Days to PMN $>0.5\times10^9$/L	Clinical outcome after UCB transplant (months)
300	M/15	70	AML 2nd CR	1	4	II	no (ABMTrescue)	59	Alive in CCR (36+)
312	F/9	34	ALL 2nd CR	1	2	Not occur.	no (autol. rec)	37	Relapse (22) 3rd CR (12+)
324	M/10	41	ALL 2nd CR	1	1	II	yes	22	Alive in CCR (29+)
325	F/4	13	ALL 2nd Re	1	4	II	yes	30	Relapse (4) Dead (10)
337	M/2	12	AML 1st CR	1	2	Not occur.	yes	56	Dead during CR (3)
341	M/6	19	ALL 2nd Rel	1	3	Not occur.	no (autol. rec.)	33	Relapse (3) Dead due to disease (4)
355	M/12	46	CML-AP	1	3	Not occur.	yes	35	Alive in CCR (14+)
359	M/6	20	ALL 2nd CR	1	1	I	yes	33	Alive in CCR (18+)
360	F/11	40	ALL 2nd Rel	2	4	I	yes	22	Alive in CCR (18+)
368	M/16	60	ALL 1st CR	2	2	I	yes	22	Alive in CCR (15+)
375	F/7	26	ALL 2nd CR	1	1	IV	no (autol. rec.)	27	Dead due to GVHD (3)
381.	M/6	28	ALL 2nd CR	1	2	III	yes	73	Dead due to sepsis
389	F/8	25	ALL 2nd CR	1	3	I	yes	33	Alive in CCR (8+)
393	F/5	20	ALL 2nd CR	1	1	I	yes	33	Alive in CR (9+)

Conv.= conventional HLA-typing. Ext.= extended HLA-typing. Autol. rec.= autologous hemopoietic reconstitution.

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twice a day for 6 doses over 3 consecutive days starting on day –7), etoposide (20 mg/kg, given as a 24-hour continuous i.v. infusion on day –4), cyclophosphamide (60 mg/kg, given as a 2-hour i.v. infusion on days –3 and –2) and antilymphocyte serum (600 IU/kg continuous infusion over 4 consecutive days starting on day –6). Prophylactic measures against GVHD consisted of daily administration of cyclosporin-A (CSA, 3 mg/kg) starting on day –1 and 6-methyl-prednisolone (2 mg/kg from day –6 to day 0 and 1 mg/kg thereafter). The dose of 6-mehylprednisolone was tapered from day 30; the full dose CSA was given until day 50 and then tapered off by 5% per week.

Engraftment and GVHD

Engraftment was established as the number of days required to recover an absolute neutrophil count of 0.5×10^9/L, and a platelet count of 20×10^9/L, in the presence of a persistent full donor chimerism status. This status was evaluated by PCR amplification of individual specific VNTR loci following the procedures previously described.[11] Donor cell engraftment and remission status were assessed on day 20, 35, 60, 100 and 1 year after transplantation.

GVHD was scored according to standard criteria.[12]

Results

From October 1995 to December 1997, 14 patients with high-risk leukemia received UCB cells from unrelated donors. As summarized in Table 2, conventional HLA-A, B and DRB1 typing showed that 12 recipient/donor pairs differed at one HLA locus (HLA A = 5 pairs; HLA B = 4 pairs; HLA DRB1 = 3 pairs), whereas 2 pairs showed the following two HLA disparities: HLA-A plus DRB1 loci: 1 case; and both the two HLA-DRB1 loci: 1 case. These 14 pairs were additionally typed for HLA-C and HLA-DQB1 genes. The extended HLA typing demonstrated additional HLA differences in 10 cases. In detail, only one out of the six pairs with a different HLA-A locus had additional mismatches at HLA-C and DQB1 loci. In contrast, the remaining 8 pairs which differed at HLA-B and/or DRB1 loci showed additional HLA-C and/or DQB1 mismatches. This difference was statistically significant by Fisher' exact test (p=0.002).

Ten out of 14 UCB recipients (71%) achieved full engraftment with complete hematologic recovery and a persistent full donor chimerism pattern. Four patients failed to engraft. Three out of these 4 cases had a transient mixed chimerism phase. Of the 4

Table 2. HLA typing of donors and recipients.

	Conventional typing			*Extended typing*		*No. of mismatched antigens*	
UPN/UCB unit	*A antigens (serologic typing)*	*B antigens (serologic typing)*	*DRB1 genes (high resolution)*	*C gene-groups*	*DQB1 genes (high resolution)*	*Standard typing*	*Extended typing*
300 / NY103433	2,24 2,24	51,44 51,44	1602,1104 1501,1104	0501,07 14,blank	0301,0502 0301,0602	1	4
312 / NY100393	1,26 1,26	8,45 8,38	0301,1302 0301,1302	07,16 07,12	0201,0609 0201,0609	1	2
324 / NY103822	1,24 24,26	38,35 38,35	0402.1104 0402,1104	04,12 04,12	0301,blank 0301,blank	1	1
325 / MI 95/R/964	1,2 1,2	50,35 8,35	1401,0701 1401,0701	0102,0602 04,07	0202,0503 0303,0503	1	4
337 / NY100492	1,2 1,2	8,40 51,8	0301,0701 0301,0701	03,07 15,07	0201,0202 0201,0202	1	2
341 / MI93/1159	2,2 2,blank	63,39 39,50	1301,0701 1301,0701	0602,07 0602,12	0202,0501 0202,0603	1	3
355 / MI95/3635	2,11 1,2	38,18 38,18	1104,1104 1104,blank	07,12 blank,12	0301,0301 0301,0603	1	3
359 / MI96/2626	11,30 3,11	7,63 7,63	1501,1302 1501,1302	07,blank 07,blank	0602,0604 0602,0604	1	1
360 / NY101788	1,3 1,3	51,7 51,7	0401,0701 0404,1104	0501,07 blank,07	0301,0303 0301,0302	2	4
368 / MI96/3699	3,24 3,11	18,35 18,35	1103,1302 1104,1302	04,07 04,07	0301,0604 0301,0604	2	2
375 / MI96/2721	1,32 1,29	52,18 52,18	1502,0302 1502,0302	12,blank 12,blank	0201,0601 0201,0601	1	1
381 / DU598	1,3 1,3	7,7 7,blank	1101,0804 1101,1501	07,blank 07,blank	0303,blank 0303,0602	1	2
389 / MI96/2618	2,29 2,29	7,44 7,44	0103,0701 0101,0701	07,16 07,blank	0202,0501 0202,0501	1	3
393 / NY103553	1,66 1,29	8,44 8,44	1501,0301 1501,0301	07,16 07,16	0601,0201 0601,0201	1	1

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patients who did not engraft, patient UPN 300 reached the defined threshold peripheral blood count by day 75, following rescue with his BM back-up. Chimerism analysis in the remaining 3 patients demonstrated early autologous hemopoietic reconstitution which was non-leukemic upon morphologic evaluation.

Acute GVHD was observed in 8 out of the 10 patients with successful engraftment, and in 2 out of the 3 patients showing a transient mixed chimerism status after UCB transplantation.

Tables 3 and 4 show an analysis of engraftment rate and GVHD occurrence according to number of mismatched HLA-antigens. In neither case was there a statistically significant differences with respect to the number of HLA-mismatches, determined by either standard or extended HLA-typing.

Discussion

HLA antigens are a highly polymorphic family of cell-surface molecules whose function is to bind and present antigenic peptides to lymphocytes, leading to initiation of the immune response.

Recently, molecular based technologies have made it possible to type alleles of several HLA genes. These approaches have contributed to the elucidation of the highly polymorphic nature of the HLA system, defining 87, 191 and 189 alleles for HLA- A, B, and DRB1 genetic loci, respectively. Furthermore, these methods allow a significantly higher resolution than that affected by serology. This is particularly important for the characterization of genetic loci whose protein products are weakly immunogenetic, for example HLA-C genes.[13] However, the high number of alleles and the wide distribution of polymorphic regions within the genes raise several technical and financial concerns about molecular HLA-A and B typing. For these reasons, molecular methods are not yet routinely used to define compatibilty of HLA class I genes.

However, several studies have recently pointed out the clinical relevance of high resolution molecular typing of HLA class I and II genes in unrelated bone marrow transplants, demonstrating that survival was significantly longer in patients receiving fully HLA-matched marrow than in those transplanted with mismatched marrow cells.[14-20]

In contrast, the degree of HLA compatibility is less relevant in UCB transplants. In fact, clinical results available in this setting have shown that UCB transplants are associated with a low risk of acute and chronic GvHD, probably as a consequence of the relative immaturity of cord blood lymphoid cells.[5] Thus, UCB searching criteria including HLA-A and B serologic typing and the possibility of selecting unrelated UCB units which differ from the potential recipients by 1 or 2 HLA antigens out of the six HLA-A, B, DRB1 antigens conventionally typed.

In the present study, we have extended conventional HLA typing to the characterization of HLA-C and DQB1 loci in 14 patients transplanted with UCB from unrelated donors in order to verify whether HLA-C and DQB1 mismatches might influence the clinical outcome of patients.

After extended HLA-typing, we found additional mismatched HLA antigens in all the 8 pairs which already differed at HLA-B and/or DRB1 loci. In contrast, only one out of the six pairs with an HLA-A mismatch had two additional differences at HLA-C and DQB1 loci. This observation, probably explained by the strong *linkage disequilibrium* existing among HLA genes, provides an additional criterion when selection of UCB donors with the closest HLA-match is requested.

Moreover, in our group of 14 patients we did not observe any correlation between engraftment rate, occurrence of acute GVHD and degree of HLA disparities even after extended typing.

Due to the limited amount of available DNA for each pair, we could not perform high resolution typing of HLA-A and B genes. However, we do believe that the lack of molecular HLA-A and B typing does not affect the interpretation of the present data. In fact, serologic typing showed that 10 out of 14 recipient/donor pairs already differed at HLA-A or B loci, while additional differences could be expected in the remaining 4 pairs following molecular typing. Thus, our conclusion that certain HLA disparities do not affect clinical outcome of patients receiving UCB transplants also holds true in the absence of HLA-A

Table 3. Engraftment rate of UCB cells according to number of mismatched antigens evaluated by conventional and extended HLA-typing.

No.of mismatched antigens	*Conventional HLA typing*		*Extended HLA typing*	
	No.of pairs	*grafted (%)*	*No.of pairs*	*grafted (%)*
1	12	8 (66%)	4	3 (75%)
2	2	2 (100%)	4	3 (75%)
3	–	–	3	2 (66%)
4	–	–		3 (66%)

Table 4. Occurrence of acute GVHD in the 13 patients with UCB engraftment (10 cases) or showing a transient mixed chimerism condition after UCB (3 cases) according to the number of mismatched antigens evaluated by conventional and extended typing.

No.of mismatched antigens	*Conventional HLA typing*		*Extended HLA typing*	
	No.of pairs	*GvHD (%)*	*No.of pairs*	*GvHD (%)*
1	11	8 (73%)	4	4 (100%)
2	2	2 (100%)	4	2 (66%)
3	–	–	2	1 (50%)
4	–	–	3	3 (100%)

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and B molecular typing.

Furthemore, should these findings be confirmed in future studies on larger series of patients, these data would suggest that expensive and time consuming molecular typing of HLA-C and DQB1 loci might be avoided during UCB donor searches.

Contributions and Acknowledgments

GC was responsible for the conception of the study, its design, ethical approval, funding, direct supervision, recruitment and contact with the participants. He also wrote the paper. LE, MT, TS performed molecular HLA-C, DRB1 and DQB1 typing. MCR perfomed the molecular analysis of chimerism. MPP performed serologic HLA-A and B typing. WA, API, CG were responsibile for the patients' care and MS was responsible for UCB unit searches. All the authors contributed to the manuscript and approved its final version. The authors wish to thank Prof. GB Ferrara for his critical reading of the manuscript.

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Disclosures

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References

1. Gluckman E, Broxmeyer ME, Auerbach AD, et al. Hematopoietic reconstitution in patient with Fanconi anemia by means of umbilical cord blood from an HLA-identical sibling. N Engl J Med 1989; 321:1174-8.
2. Wagner JE, Kernan NA, Steinbuch M, Broxmeyer HE, Gluckman E. Allogeneic sibling umbilical cord-blood transplantation in children with malignant and non malignant disease. Lancet 1995; 346:214-9.
3. Wagner JE, Rosenthal J, Sweetman R, et al. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease. Blood 1996; 88:795-02.
4. Kurtzberg J, Laughlin M, Graham ML, et al. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med 1996; 335:157-66.
5. Gluckman E, Rocha V, Boyer-Chammard A, et al. Clinical outcome of cord blood transplantation from related and unrelated donors. N Engl J Med 1997; 337:373-81.
6. Locatelli F, Burgio GR. Transplant of hematopoietic stem cells in childhood: where we are and where we are going. Haematologica 1998; 83:550-63.
7. Miller SA, Dykes DD, Polesky HF, et al. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Research 1988; 16:1215.
8. Terasaki P. Microdroplet assay for human blood lymphotoxins. In: [anonymous]. Hystocompatibility testing. National Academy of Sciences, Washington, D.C. 1965. p. 171.
9. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 1992; 39:225-35.
10. Bodmer JG, Marsh SGE, Albert ED, et al. Nomenclature for factors of HLA system, 1996. Tissue Antigens 1997; 49:297-321.
11. Rapanotti MC, Arcese W, Buffolino S, et al. Sequential molecular monitoring of chimerism in chronic myeloid leukemia patients receving donor lymphocyte transfusion for relapse after bone marrow transplantation. Bone Marrow Transplant1997; 19:703-7.
12. Przepiorka D, Weisdorf D, Martin P, et al. 1994 consensus conference on acute GVHD grading. Bone Marrow Transplant 1995; 15:825-8.
13. Zemmour J, Parham P. Distinctive polymorphism at the HLA-C locus: implication for the expression of HLA-C. J Exp Med 1992; 176:937-50.
14. Speiser DE, Tiercy JM, Rufer N, et al. High resolution HLA matching associated with decreased mortality after unrelated bone marrow transplantation. Blood 1996; 87:4455-62.
15. Petersdorf EW, Longton GM, Anasetti C, et al. Association of HLA-C disparity with graft failure after marrow transplantation from unrelated donors. Blood 1997; 89:1818-23.
16. Petersdorf EW, Longton GM, Anasetti C, et al.The significance of HLA-DRB1 matching on clinical outcome afterHLA-A, B,DR identical unrelated donor marrow transplantation. Blood 1995; 86:1606-13.
17. Petersdorf EW, Stanley F, Martin P J, Hansen A. Molecular diversity of the HLA-C locus in unrelated marrow transplantation. Tissue Antigens 1994; 44:93-9.
18. Davies SM, Shu OX, Blazar BR, Filipovich AH, et al. Unrelated donor bone marrow transplantation: influence of HLA A and B incompatibility. Blood 1995; 86:1636-42.
19. Nagler A, Brautbar C, Slavin S, et al. Bone marrow transplantation using unrelated and family related donors: the impact of HLA-C disparity. Bone Marrow Transplant 1996;18:891-7.
20. Arcese W, Aversa F, Bandini G, et al. Clinical use of allogeneic hematopoietic stem cells from sources other than bone marrow. Haematologica 1998; 83:159-82.

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