Multiple Minor Histocompatibility Antigen Disparities Between a Recipient and Four HLA-Identical Potential Sibling Donors for Bone Marrow Transplantation

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ABSTRACT: A patient with acute leukemia and her family including four HLA-identical siblings were analyzed to select a donor who was not only HLA- but also minor histocompatibility (mH) antigen compatible for allogeneic bone marrow transplantation (BMT) The HLA-A2 restricted mH antigen-specific HA-1, -2, -4, and -5 cytotoxic T-lymphocyte (CTL) clones were used to type the family members for expression of these mH antigens The patient and one HLA-identical sibling were compatible for these mH antigens This sibling was selected as the bone marrow donor The patient engrafted promptly but developed acute and chronic graft-versus-host disease To study the presence of other mH antigen disparities between recipient and donor, host-versus-graft CTL lines and clones were generated by stimulation of recipient peripheral blood lymphocytes (PBLs) with donor bone marrow cells, and graft-versus-host CTL lines were generated after BMT by stimulation of PBLs of donor origin with recipient bone marrow cells These CTL lines were cytotoxic to cells from the bone marrow donor and from the recipient, respectively, and to cells from several other family members T-cell lines, generated from the patient after BMT by stimulation of recipient-derived PBLs with donor bone marrow cells, exhibited no specific cytotoxicity to donor or recipient cells Chimerism studies after BMT revealed that the PBLs and T-cell lines generated after BMT were of donor origin CTL lines that were generated from PBLs from the three other HLA-identical siblings in this family by stimulation with HLA-identical donor bone marrow cells also exhibited cytotoxicity to cells from several family members Our results show that in addition to compatibility for HA-1, -2, -4, and -5 between the recipient and the donor, other mH antigen disparities existed between all HLA-identical siblings, illustrating the high degree of polymorphism of mH antigens and therefore the difficulty of finding mH antigen-compatible donor–recipient pairs even when more than one HLA-identical sibling is present within a family *Human Immunology 37, 221–228 (1993)*

ABBREVIATIONS

aGvHD	acute graft-versus-host disease
BMMNC	bone marrow mononuclear cell
BMT	bone marrow transplantation
CTL	cytotoxic T lymphocyte
EBV	Epstein–Barr virus
GvH	graft versus host
HvG	host versus graft
IL-2	interleukin 2
LCL	lymphoblastoid cell line
Leuko-A	leukoagglutinin-A
mH	minor histocompatibility
MHC	major histocompatibility complex
PCR	polymerase chain reaction
PBL	peripheral blood lymphocyte
PHA	phytohemagglutinin
RFLP	restriction fragment-length polymorphism
TCGF	T-cell growth factor

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INTRODUCTION

HLA-identical bone marrow transplantation (BMT) is frequently complicated by acute graft-versus-host disease (aGvHD). This complication is thought to be induced by donor T cells from the graft that specifically

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TABLE 2 Reactivity of recipient derived α donor bone marrow CTL lines and clones

Effector cells	Specific lysis in ^{51}Cr release assay of IL 2 blasts from: Donor ac[b]	Rec ac	Sib 3 ac	Sib 4 ac	Sib 5 ac	Sib 6 ad	Sib 7 ad	Sib 8 bd	Mother cl
CTL line 1[a]	+++	–		+++	–		+++		
CTL line 1 A	+++	–	+++	+++	+++		+++		+++
CTL line 1 B	++		++	++	++	–			
Clone 10F8[d]	+++	–	–	+++	–		+++		

[a] Effector–target ratio 20 1
[b] See Table 1 notes
See Table 1 notes
[d] Effector target ratio 5 1

clones with the same specificity CTL clone 10E8 is a representative example of these clones that recognized cells from the bone marrow donor and from siblings 4 and 7 Phenotypic analysis of the clones by FACS scanning, using anti-CD3, -CD4, and CD8 monoclonal antibodies (Becton-Dickinson, Mountain View CA USA) showed that they were $CD3^+CD8^+$ These results show that CTL lines can be generated with various mH antigen specificities from pre BMT recipient PBLs by stimulation with donor BMMNCs

Recognition of cells from family members by GvH CTL lines Table 3 shows that the CTL lines generated from PBLs collected at several intervals after BMT and stimulated with irradiated recipient pretransplant BMMNCs differentially recognized cells from other family members, which suggested the presence of different mH antigen specificities in the GvH CTL lines

Recognition of cells from family members by the sibling 3 4 and 5–antidonor CTL lines CTL lines were generated from PBLs from three other HLA identical siblings by stimulation with irradiated donor BMMNCs Table 4 shows that these CTL lines differentially recognized cells from other family members CTL line 4–antidonor only lysed to a limited extend EBV LCLs from the donor but not IL 2 blasts

Lack of donor specific recognition by post BMT antidonor bone marrow T cell lines To investigate the presence of residual potential donor directed CTLs in the peripheral blood of the recipient after BMT PBLs collected on days +14 +19 +27 +40 +60 and +180 were stimulated with irradiated donor BMMNCs T cell lines generated from PBLs collected on days +14 +19 +27 and +40 were neither cytotoxic for cells from the recipient nor for cells from the bone marrow donor Cytotoxicity against EBV LCLs but not IL 2 blasts from recipient and bone marrow donor were detected in the T cell lines generated from PBLs collected on days +60 and +180 and that had been stimulated with donor BMMNCs only The observed cytotoxicity was not donor specific however since autologous cells also were recognized (data not shown) Determination of the origin of the T cell lines generated after BMT showed that they all were donor derived (Fig 1)

TABLE 3 Reactivity of GvH CTL lines

Effector cells[a]	Specific lysis in ^{51}Cr release assay of IL 2 blasts from: Donor ac[b]	Rec ac	Sib 3 ac	Sib 4 ac	Sib 5 ac	Sib 6 ad	Sib 7 ad	Sib 8 bd	Mother cl
CTL line +28	–	++	+++	+++	+++	+	+	++	+
CTL line +105	–	+	++			–		+	
CTL line +183	–	+	++	++			–	+	+
CTL line +365	–	++	+	+	–		–	+	

[a] Effector–target ratio 20 1
[b] See Table 1 notes
See Table 1 notes

TABLE 4 Reactivity of sibling 3, 4, and 5–antidonor CTL lines

	Specific lysis in ^{51}Cr release assay of cells from										
Effector cells	Donor IL 2 blasts ac[a]	EBV LCLs ac	Rec IL 2 blasts ac	EBV LCLs ac	Sib 3 IL 2 blasts ac	Sib 4 IL 2 blasts ac	Sib 5 IL 2 blasts ac	Sib 6 IL 2 blasts ad	Sib 7 IL 2 blasts ad	Sib 8 IL 2 blasts bd	Mother IL 2 blasts cd
CTL line 3 ∝ donor	+ +	+ + +		+ + +				+ + +	ND[b]	+ + +	+ + +
CTL line 4 ∝ donor		+				–			ND		
CTL line 5 ∝ donor	+ +	+ + +		+ + +	–			+	ND	+ +	

Effector:target ratio 20:1

[a] See Table 1 notes

[b] See Table 1 notes

[c] Not done

DISCUSSION

HLA identical BMT may be complicated by severe aGvHD or graft rejection despite optimal matching for HLA class I and II antigens [1–7]. Both complications are thought to be the result of the recognition of mH antigens by immunocompetent T lymphocytes from donor or recipient origin, respectively [2, 13]. Minor histocompatibility antigens are thought to be peptides derived from cellular proteins [26] that are bound in the groove of major histocompatibility complex (MHC) molecules. This MHC molecule–peptide complex can be recognized by alloreactive T lymphocytes [27]. Consequently, matching for mH antigens between donor and recipient may decrease the incidence and severity of aGvHD and graft rejection.

In mice, more than 50 mH genes have been described [28]. In humans, several authors have described mH antigen specific CTLs in patients after multiple blood transfusions and bone marrow and kidney transplantation [2, 29–32]. Mapping of the gene loci has been successful only for the male mH antigen H-Y, both in humans and mice [33, 34], and for MTF, a mouse mH antigen encoded by mitochondrial DNA [35]. If the polymorphism of immunogenic human mH antigens is as extended as in mice, the chances of finding donor–recipient pairs identical for both major and minor histocompatibility antigens may be limited.

Previously, we have shown that, prior to BMT, recipient–antidonor cytotoxicity was demonstrated in seven of ten HLA-identical donor–recipient pairs [21]. In the present study, we show that although donor and recipient were matched for the mH antigens HA-1, -2, -4, and -5, additional cytotoxic reactivity against other, as yet undefined, mH antigens was present between these two siblings, both in the GvH and the HvG directions. The differences in recognition patterns between the HvG parent line and its sublines and between the GvH lines generated at different time intervals after BMT

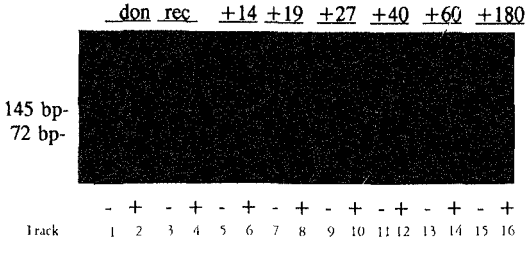

FIGURE 1 RFLP analysis adapted for PCR amplification, of PBLs from the bone marrow donor and the recipient, and of the posttransplant antidonor bone marrow T cell lines. The *bands* represent PCR amplified DNA. *Tracks* marked + are digested with the restriction enzyme AvaII and tracks marked – are undigested. *Tracks 1 and 2* are donor cell derived and produce only one band, whereas *tracks 3 and 4* are recipient cell derived and produce two bands when digested with AvaII (track 4). Recipient and donor cells were collected before BMT. *Tracks 5–16* are derived from recipient post BMT antidonor BMMNC T cell lines, generated from recipient PBLs collected 14–180 days after BMT.

may reflect differences in the frequencies of the various mH antigen-specific CTL clones present in the CTL lines The differential reactivity of the post-BMT GvH CTL lines may reflect in vivo sensitization of donor T cells against mH antigens These mH antigens may be sequentially expressed on recipient stimulator cells, e g , due to sequential infections by pathogens that upregulate expression of various self-mH antigens In addition, when PBLs from the three other HLA-identical siblings were stimulated with irradiated donor BMMNCs, CTL lines were generated that also recognized mH antigens expressed on cells from family members These results illustrate multiple mH antigen disparities between the HLA-identical family members

Despite the presence of host–antidonor cytotoxic reactivity prior to BMT, no graft rejection occurred No residual recipient cells were demonstrated in peripheral blood and bone marrow collected from the recipient by chimerism studies after BMT These findings were in accordance with the inability to generate recipient–antidonor CTL lines from posttransplant PBLs, suggesting that no functional alloreactive recipient T lymphocytes were present after BMT These findings can be explained by the eradication of recipient-immunocompetent T cells by the conditioning regimen [36, 37], by their suppression by T cells from the graft [3], or by the posttransplant immunosuppressive therapy [38] Uncomplicated engraftment despite in vitro HvG reactivity prior to BMT agrees with a previous report [21] in which we describe that, in seven of ten HLA-identical donor–recipient pairs, recipient–antidonor CTL lines were generated before BMT None of these patients showed signs of graft failure or graft rejection

GvHD was not prevented by matching for expression of the mH antigens HA-1, -2, -4, and -5, suggesting that also in the GvH direction additional mH antigen disparities were present between donor and recipient Our in vitro results with T-cell lines, generated by stimulation of PBLs collected at several intervals after BMT with recipient pre-BMT BMMNCs, paralleled the in vivo observed GVH reactivity Van Els et al [39] could not demonstrate a clear correlation between the incidence of GvHD and the ability, or inability, to generate antihost CTL lines after BMT Together the results reported here and those by Van Els et al indicate that most donor–recipient pairs are mH antigen disparate, but that there is not necessarily a relation between the ability, or inability, to generate CTL lines that lyse recipient or donor cells and the occurrence of GvHD or graft rejection

Presently, matching for mH antigens is not feasible due to their number and polymorphism Quantitative, more than merely qualitative, differences in mH antigen-specific T-cell responses between recipients and their potential donors may be correlated with graft rejection or GvHD Quantitative differences may be estimated by using limiting dilution assays to determine CTL or T-helper-cell precursor frequencies as has been described in unrelated HLA-matched donor–recipient pairs or allogeneic responder–stimulator pairs by Kaminski et al [40, 41] and Deacock et al [42], respectively Adapting these protocols to the specific situation of HLA-identical sibling BMT, e g , by using BMMNCs as stimulator cells, may produce valuable information

In conclusion, we have analyzed the mH antigen disparity between HLA-identical and -nonidentical family members of a patient with leukemia, and the possibility of finding a sibling bone marrow donor who was both HLA and mH antigen compatible In addition to compatibility for the mH antigens HA-1, -2, -4, and -5 between bone marrow donor and recipient, disparity for multiple other mH antigens was demonstrated both in the HvG and the GvH directions CTL lines were generated from PBLs from three other HLA identical siblings by stimulation with donor BMMNCs, also recognizing mH antigens expressed on cells from family members Donor–antirecipient, but no residual recipient–antidonor, reactivity was demonstrated at several intervals after BMT In view of these findings, finding donor–recipient pairs that are HLA and mH antigen identical is unlikely Quantitative, more than merely qualitative, differences in the immune responses to mH antigens between recipients and their potential donors may more likely determine the incidence or severity of GvHD or graft rejection following HLA-identical BMT

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