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Are changes in HLA Ags responsible for leukemia relapse after HLA-matched allogeneic hematopoietic SCT?

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Abstract

Loss of heterozygosity (LOH) has been shown to be associated with leukemia relapse after haploidentical transplantation. Whether such changes are an important cause of relapse after HLA-matched transplantation remains unclear. We retrospectively HLA-typed leukemic blasts for 71 patients with AML/myelodysplastic syndrome obtained from stored samples, and the results were compared with those obtained at diagnosis and/or before the transplant. No LOH or any other changes in HLA Ag were found in any of the samples tested post transplant as compared with pretransplant specimens. One patient had LOH in HLA class I Ag (HLA-A,-B and -C); however, these changes were present in the pretransplant sample indicating that they occurred before the transplant. We concluded that, in contrast with haploidentical transplantation, HLA loss does not have a major role as a mechanism of relapse after allogeneic transplantation with a closely HLA-matched donor.

INTRODUCTION

Disease relapse after allogeneic hematopoietic SCT (allo-HSCT) is the most important cause of treatment failure. The mechanisms of leukemia relapse after allo-SCT remain elusive.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

AH collected data and wrote the paper; MAFV and KC contributed to study design, data collection and interpreted results; LMP and FA contributed to data collection and interpretation of results; SK performed the blast concentration; REC contributed with interpretation of the results and manuscript writing; SOC contributed with study design, data collection, interpretation of the results and manuscript writing. All authors critically reviewed and approved the manuscript.

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Loss of HLA class I Ags has been associated with disease relapse, tumor progression and metastasis in solid tumors.[1–5] In addition, loss of heterozygosity (LOH) has been shown to be a common cause of disease relapse after T-cell-depleted haploidentical transplantation.[6,7] It is believed that these changes are a direct consequence of selective immune pressure mediated by T cells.[8–10] Here we aimed to determine if changes in HLA Ags occur after closely HLA-matched transplants and can be responsible for leukemia relapse post transplant.

MATERIALS AND METHODS

Patients and transplantation procedure

We included all patients with AML or myelodysplastic syndrome who received an HLA-matched related or a closely HLA-matched unrelated donor transplant at our institution between March 1997 and August 2009, who relapsed after HSCT and had samples stored in the institutional sample bank. HLA typing was performed for all patients' samples at the time of the initial diagnosis, pretransplant and post relapse, either prospectively or on archived cryopreserved samples and for all donor samples. We compared the HLA typing for the post-relapse specimens with the specimens HLA typed at diagnosis and/or pretransplant and aimed to evaluate whether LOH occurred in the leukemic blasts at the time of relapse. This study was approved by Institutional Review Board, and a waiver of informed consent was obtained to analyze the archived samples. Written informed consent was obtained for treatment at our institution from all patients in accordance with the Declaration of Helsinki.

Sample collection and cryopreservation

Blood and BM aspirates underwent ficoll separation to remove RBC and neutrophils, leaving a mononuclear cell population along with B and T cells. We then performed a magnetic depletion of the B and T cells using beads for CD3+ and CD19+ cells using a MACS device (Miltenyi, Auburn, CA, USA), to yield a leukemia-enriched blast population. Cells were cryopreserved in Roswell Park Memorial Institute media supplemented with 20% FCS +10% DMSO and frozen using a programmed cell freezer. Contaminating B and T cells were <2% after the depletion. A validated methodology which detected leukemic cells >90% of the remaining material.

DNA isolation and HLA typing

Genomic DNA from malignant cells was extracted utilizing the QIAsymphony DNA Investigator Kit (QIAGEN, Valencia, CA, USA). HLA typing was performed for HLA-A, -B, -C, -DRB1 and -DQB1 loci by PCR amplification and oligonucleotide hybridization using molecular methods of the LABType SSO kit from One Lambda (Canoga Park, CA, USA), this is a reverse sequence-specific oligonucleotide hybridization in which the DNA is amplified by PCR and then hybridized with the bead probe array. The array platform uses microspheres as a solid support to immobilize oligonucleotide probes. Hybridization to each fluorescent bead is detected in a Luminex 100/200 System (Luminex Corporation, Austin, TX, USA).

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HLA typing was performed for all samples at intermediate and high resolution for HLA-A, -B, -C, -DRB1 and -DQB1 loci by PCR amplification and oligonucleotide hybridization using molecular methods and commercial kits from Invitrogen (Carlsbad, CA, USA), ELPHA (Dreieich, Germany) and/or One Lambda (Canoga Park, CA, USA) that achieved intermediate resolution. The patients and donors were also typed prospectively for these loci by high-resolution methods (PCR amplification and nucleotide sequencing) using SEQR Sequence Based Typing Kits (Abbott Park, IL, USA).

RESULTS AND DISCUSSION

We identified 71 patients with a median age of 51 years (range 18–67) who met the inclusion criteria. Forty-eight percent of the patients were males. The majority of patients had AML/myelodysplastic syndrome relapse after a 10/10 allele matched HSCT ($N = 57$, 80%). Of 14 patients relapsed after mismatched HSCT, 12 had one allele mismatched at HLA class I or II loci (9/10, 17%) and two had two alleles mismatched (8/10, 3%). The patients' characteristics are summarized in Table 1. Conditioning consisted of a myeloablative regimen in 46 patients (65%) and reduce-intensity regimen in 25 patients (35%). Twenty-six patients (36%) were in remission at the time of transplant, whereas the rest had active disease. Forty-seven (66%) had peripheral blood and 24 patients (34%) had BM as the stem cell source. Out of the 71 patients tested, LOH at HLA class I loci (HLA-A, -B and -C) in relapsed blasts was present in only one case after transplant (Figure 1). In this patient who was a 58-year-old female with myelodysplastic syndrome who underwent 9/10 matched unrelated donor transplant there was loss of HLA-A02:05, B14:02:01 and C08:02 and her AML blasts became homozygous at these loci (Figure 1). However, these changes were also observed in the blasts collected in a pretransplant specimen, suggesting that the LOH happened before transplantation. No other HLA loss was observed in any other sample of studied patients.

The rate of HLA loss is low in hematologic malignancies compared with solid tumors.[11–13] Previous studies have suggested that loss or downregulation of HLA class I may have an important role in relapsed leukemia and may be involved in relapse after HSCT or failure of DLI. The report by Masuda *et al.*[14] showed that the loss or downregulation of HLA class I in acute leukemia blasts occurs more frequently in relapse compared with the initial diagnosis (2/5, 40% vs 3/39, 7.7%). However, they did not examine and compare the expression of HLA class I alleles at the initial diagnosis and relapse. Other investigators described pretransplant loss or downregulation of HLA class I in hematologic malignancies patients.[15–20] Dubois *et al.*[21] reported six patients with LOH in HLA region due to uniparental disomy before HSCT. In a study of 600 HSCT patients with myeloid malignancies, Pereira *et al.*[19] showed pretransplant loss of HLA class I in three cases. Also, loss of HLA class I through acquired uniparental disomy has been described in relapsed leukemic blasts after haploidentical HSCT.[6,7] Smith *et al.*[20] reported somatic mutations in HLA gene of 15 patients who underwent HSCT for hematological malignancies. Thirteen of these patients were found to have the mutation in pretransplant samples, whereas in 2 patients, mutations were detected in post-transplant relapse specimens. One was an ALL patient who received 1 Ag-mismatch unrelated donor transplant and the other one was a CML patient who received a haploidentical transplant. There is only one case report in the

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literature of HLA loss in a relapsed AML after matched unrelated donor HSCT.[22] It is, however, unclear if this was present immediately before transplant or occurred after the transplant, as we were fortunate to determine in our case.

Several mechanisms have been proposed for such HLA class I alterations including: absence of β2-microglobulin[4] or peptide transporter expression[23–25] LOH, large deletions or mitotic recombination in chromosome 6[25–28] transcriptional downregulation[29] or point mutation, partial deletion or somatic recombination.[25,30,31]

In a series of 77 patients with relapse after mismatched transplantation for high-risk myeloid malignancies, no HLA loss among 11 mismatched unrelated donor transplants was identified.[32] Also, in a series of nine pediatric patients with acute leukemia, Villalobos *at al*.[7] found no HLA loss in post-HSCT leukemic blasts of six patients with matched HSCT. Our findings are consistent with these results and, although our methodology was not able to detect downregulation in HLA Ags, we showed that loss of HLA Ags may not have a major role in disease recurrence after a closely HLA-matched transplant. Further studies evaluating the alteration in HLA Ags post transplant are needed to determine if these changes are the result of immunologic pressure related to transplant or occurred just before transplantation and were identified only at the time of relapse.

In conclusion, in contrast to frequent finding of LOH in leukemic blasts after haploidentical transplantation and/or donor T-cell infusion, LOH was not identified in leukemic blasts at the time of relapse in T-cell-replete matched related or unrelated donor transplants. These results suggest that LOH could be a particular mechanism for leukemia relapse after haploidentical transplantation, whereas it does not appear to be a mechanism for leukemia relapse after allogeneic transplantation with a closely HLA-matched donor.

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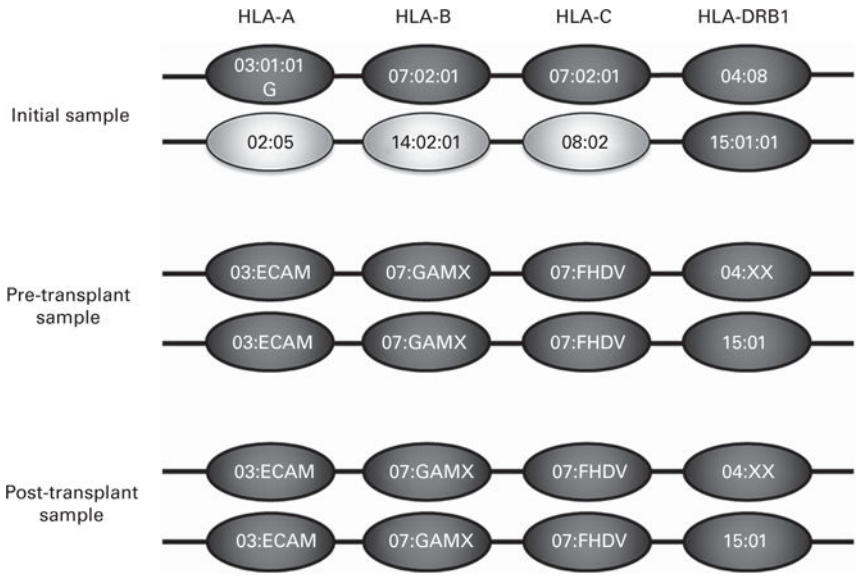

Figure 1.
LOH in HLA class I loci (HLA-A, -B, -C) in pretransplant relapsed blasts in one patient.

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Table 1

Patients and transplants characteristics

Characteristic	*Number (%)*
Age, median range	51, 18–67
Sex	
Male	34 (48%)
Female	37 (52%)
Status at SCT	
CR1	11 (15%)
Primary induction failure	17 (24%)
CR2	15 (21%)
Active disease	28 (39%)
Cytogenetic risk group	
Good	7 (10%)
Intermediate	35 (49%)
Poor	27 (38%)
Unknown	2 (3%)
Donor type	
Matched	57 (80%)
1 Ag mismatched	12 (17%)
HLA-A	7
HLA-C	1
HLA-DRB1	1
HLA-DQB1	3
2 Ag mismatched	2 (3%)
HLA-A/HLA-C	1
HLA-A/HLA-DRB1	1
Donor relation	
Related	36 (51%)
Unrelated	35 (49%)
Stem cell source	
PB	47 (66%)
BM	24 (34%)
Conditioning regimen	
Myeloblative	46 (65%)
Reduced intensity	25 (35%)

Abbreviation: PB = peripheral blood.