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Therapeutic and Diagnostic Applications of Minor Histocompatibility Antigen HA-1 and HA-2 Disparities in Allogeneic Hematopoietic Stem Cell Transplantation: A Survey of Different Populations

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ABSTRACT

Minor histocompatibility antigens (mHags) HA-1 and HA-2 are encoded by biallelic loci, with immunogenic variants, HA-1^H and HA-2^V, which induce strong HLA-A2–restricted alloreactive T-cell responses, and nonimmunogenic counterparts, HA-1^R and HA-2^M, which represent functional null alleles that are poorly presented by HLA class I molecules. HA-1 and HA-2 are potential targets of selective graft-versus-leukemia and graft-versus-tumor reactivity after allogeneic hematopoietic stem cell transplantation (HSCT); however, these applications are restricted to a limited number of patients. Here, we show that a far more frequent application of HA-1 and HA-2 disparity relies on their use as markers for the state of host chimerism after allogeneic HSCT. We have determined allelic frequencies of 29.3% and 70.7% for HA-1^H and HA-1^R, respectively, and of 83.7% and 16.3% for HA-2^V and HA-2^M, respectively, in >200 healthy individuals from northern Italy. Similar frequencies were observed in nearly 100 patients affected by hematologic malignancies or solid tumors, thus showing that HA-1 and HA-2 variability are not associated with the presence of cancer. On the basis of these data, we predict that HA-1 and HA-2 can be used in 32.8% and 23.5% of Italian transplant patients, respectively, as markers for the state of host chimerism, whereas exploitation of disparity for these mHags for targeted immunotherapy will be possible in 10.7% and 1.1% of Italian patients, respectively. Retrospective HA-2 typing of bone marrow aspirates obtained from a patient during complete remission or recurrence of acute myeloid leukemia after haploidentical HSCT showed the feasibility of using HA-2 as a surrogate marker for disease monitoring. Because of an apparent north-south gradient for HA-1 allelic frequencies, with higher frequencies for the HA-1^H variant reported in white populations from Southern Europe as compared with Northern Europe and North America, the diagnostic applicability of HA-1 disparity will be slightly more frequent in transplant patients from the north. Taken together, our data show that determination of HA-1 and HA-2 variability can be an important parameter for the selection of allogeneic stem cell donors, in particular for patients affected by hematologic malignancies without a tumor-specific molecular marker.

KEY WORDS

Minor histocompatibility antigens HA-1 and HA-2 • Hematopoietic stem cell transplantation • Minimal residual disease • Host chimerism • Graft-versus-leukemia activity • Population study

INTRODUCTION

Minor histocompatibility antigens (mHags) are peptides derived from largely undefined polymorphic proteins encoded throughout the human genome and presented by self major histocompatibility complex molecules to alloreactive T cells [1]. The mHags play an important role in allogeneic hematopoietic stem cell transplantation (HSCT) because they are the only targets of graft-versus-host disease in HLA-matched sibling or unrelated donor transplantation, and they contribute to this major clinical complication also after partially HLA-mismatched HSCT [2,3]. Conversely, mHags are also responsible for the immunotherapeutic effect mediated by donor T cells after allogeneic HSCT, both against leukemia (graft versus leukemia; GVL) and against solid tumors (graft versus tumor; GVT) [3-6]. Considerable effort has been spent on trying to separate graft-versus-host disease from GVL/GVT. One approach to tackle this challenge relies on the identification of mHags selectively expressed in tissues of hematopoietic origin but absent in other cell types. Particular interest has been raised by 2 mHags that fulfill these criteria, HA-1 and HA-2: both are encoded by biallelic gene systems with one immunogenic and a second nonimmunogenic variant [7,8]. In particular, the immunogenic peptides encoded by HA-1^H and HA-2^V are presented on the cell surface by HLA-A2 molecules and recognized by HLA-A2–restricted cytotoxic T cells, whereas the nonimmunogenic variants HA-1^R and HA-2^M are functionally silent because of poor presentation by HLA class I molecules. HA-1, which is expressed not only on hematopoietic cells, but also in a variety of solid tumors (and, hence, is a potential target of GVT [9]), encodes a protein of unknown function [10,11]. The protein encoded by HA-2 belongs to the family of myosins involved in formation of the cell cytoskeleton [12].

HA-1 and HA-2 are potential targets of immunotherapy in patients who carry HLA-A2 and at least 1 immunogenic mHag variant after transplantation from a donor homozygous for the nonimmunogenic variant [13-18]. In such patients, tracing of HA-1–specific cytotoxic T lymphocytes by tetramer staining has been successfully used to monitor GVL activity after transplantation [14]. However, this important application is limited to a restricted number of patients because of the allelic frequencies of HA-1 and HA-2 variants [19]. Recently, HA-1 has been used as marker for maternal-fetal microchimerism in individuals sensitized to HA-1 by pregnancy or multiple blood transfusions [20]. We show here that a far more frequent application of HA-1 and HA-2 disparity after allogeneic HSCT relies on the use of these 2 mHags as molecular markers for the state of host chimerism (HC). By systematic comparison of HA-1 and HA-2 allelic frequencies in Italy with those previously reported for other populations [10,11,19,21-25], we demonstrate the presence of a north-south gradient for the frequencies of HA-1 variants. This gradient determines a slightly higher diagnostic applicability of HA-1 disparity for patients from Northern Europe and North America as compared with Mediterranean countries.

MATERIALS AND METHODS

Study Population

A total of 208 and 199 healthy blood donors from northern Italy were typed for HA-1 and HA-2 polymorphisms, respectively. A total of 99 Italian patients who underwent allogeneic HSCT from HLA-identical (n = 59) or haploidentical (n = 33) family donors or from HLA-identical unrelated donors (n = 7) for hematologic malignancies (n = 82) or solid tumors (n = 17) were also included in the study. All 99 patients were typed for HA-1 variants, and HA-2 typing was performed on 91 of these patients, 74 of whom had hematologic malignancies. One patient, patient 20, was heterozygous for the 2 HA-2 variants and received a transplant from an HA-2^V homozygous haploidentical family donor for acute myeloid leukemia. This patient experienced initial complete disease remission (CR) after transplantation (CR1) but experienced disease relapse at day +150 after transplantation as evidenced by morphologic analysis (23% leukemic blasts). Bone marrow aspirates from this patient at day +90 (CR1) and +150 (relapse) were retrospectively typed for HA-2 by using the sequence-specific polymerase chain reaction (PCR) amplification, as described below.

Genomic Typing of HA-1 and HA-2 Polymorphisms

Genomic DNA was extracted from 210 μL of peripheral ethylenediaminetetraacetic acid-blood by using a commercial DNA-extraction kit (Sigma, St. Louis, MO). HA-1 and HA-2 typing by PCR-based sequence-specific priming was performed according to previously published protocols [10,26], with slight modifications. These modifications consisted of the use of only 1 primer mix for each variant and the inclusion of primers for amplification of HLA-DRB [27] or HLA-DPB1 [27] as an internal control for each reaction (Table 1). PCR conditions were identical for the amplification of HA-1 and HA-2; thermal cycler programs were used as described previously [10,26]. PCR reactions were performed in 25 μL of a reaction mixture that contained 5 pmol of each HA-1–specific or HA-2–specific primer, 2.5 pmol of internal control primer, 80 ng of genomic DNA, 5× E-Buffer (Invitrogen, NV Leek, The Netherlands), 0.25 mmol/L deoxynucleoside triphosphates (Invitro-

Table 1. *Primer Pairs Used in This Study for HA-1 and HA-2 Genomic Typing*

Primer	Nucleotide Sequence (5′-3′)	Position
HA1Cfor*	GTGCTGCCTCCTGGACACTG	Exon A
HA1Hrev*	TGGCTCTCACCGTCATGCAG	Exon A/ intron
HA1Rrev*	TGGCTCTCACCGTCACGCAA	Exon A/ intron
HA2Cfor†	ACAGTCTCTGAGTGGCTCAGC	Intron
HA2Vrev†	CAGCTCCTGGTAGGGGTTCAC	Exon
HA2Mrev†	CAGCTCCTGGTAGGGGTTCAT	Exon
DRBfor‡	CGTGTCCCCACAGCACGTT	Exon 2
DRBrev‡	CCGCTGCACTGTGAAGCT	Exon 2
DPBAmpA§	GAGAGTGGCGCCTCCGCTCAT	Intron 1
DPBAmpB§	GCCGGCCCAAAGCCCTCACTC	Intron 2

*Primer sequence was reported previously [10].

†Primer sequence was reported previously [26].

‡Primer for amplification of HLA-DRB as an internal control for HA-1 typing [27].

§Primer for amplification of HLA-DPB1 as an internal control for HA-2 typing [27].

gen), 2 mmol/L $MgCl_2$ (Applied Biosystems, Foster City, CA), and 0.6 U of AmpliTaq DNA polymerase (Applied Biosystems). Amplicons were separated by 2% agarose gel electrophoresis and yielded specific bands of 190 and 274 base pairs (bp) for HA-1 and HA-2, respectively. Internal control bands had a size of 276 bp for HLA-DRB and of 327 bp for HLA-DPB1. An HA-2–typed B-lymphoblastoid cell line, generously provided by Els Goulmy, Leiden, The Netherlands, was used as a reference for the homozygosity of $HA\text{-}2^M$.

Calculation of the Probability of Using HA-1 or HA-2 Disparity for Targeted Immunotherapy after Allogeneic HSCT

The probability of being able to exploit HA-1 or HA-2 disparity for GVL activity after allogeneic HSCT was calculated on the basis of allelic frequencies for the immunogenic and the nonimmunogenic variants of these 2 mHags and of the reported phenotypic frequencies for HLA-A2 [28] in different populations. The prediction was based on the concept that for targeted immunotherapy, the patient (and, in HLA-identical HSCT, consequently also the donor) must express the HLA-A2 restriction element and at least 1 allele that encodes the immunogenic variants $HA\text{-}1^H$ or $HA\text{-}2^V$. In contrast, the donor must be homozygous for the nonimmunogenic alleles because the presence of the immunogenic variants leads to clonal deletion of self-reactive mHag-specific T cells [29]. On the basis of these considerations, the percentage *P*-GVL of patients in whom a GVL effect against the immunogenic $HA\text{-}1^H$ and $HA\text{-}2^V$ variants could be exploited was calculated according to the formula

$$p\text{-}GvL = \left[(\mathrm{pNI} + \mathrm{pII}) \times \mathrm{pNN} \times \mathrm{pA2}\right] \times 100,$$

where pNI indicates the frequency of heterozygosity for the nonimmunogenic (N) and the immunogenic (I) mHag variant; pII indicates the frequency of homozygosity for the immunogenic (I) mHag variant; pNN indicates the frequency of homozygosity for the nonimmunogenic (N) mHag variant, and pA2 indicates the phenotypic frequency for HLA-A2 [28].

Calculation of the Probability of Using HA-1 or HA-2 Disparity as a Marker for the State of HC after Allogeneic HSCT

This application requires the presence in the patient of an HA-1 or HA-2 variant not shared by the donor and is independent from the expression of HLA-A2 and from the presence or absence of an immunogenic mHag variant. The percentage *P*-HC of patients predicted in whom this diagnostic application could be exploited was calculated according to the formula

$$p\text{-}HC = \left[\mathrm{pII} \times (\mathrm{pNI} + \mathrm{pNN}) + \mathrm{pNN} \times (\mathrm{pNI} + \mathrm{pII})\right] \times 100,$$

where the variables are as described previously.

RESULTS

Allelic Frequencies of HA-1 in the Italian Population

Genomic typing of HA-1 was applied to 208 healthy blood donors from northern Italy. The allelic frequency of $HA\text{-}1^H$ and $HA\text{-}1^R$ was 29.3% and 70.7%, respectively (Table 2). To determine whether HA-1 variability is associated with the presence of cancer, 99 Italian patients affected by hematologic malignancies (n = 82) or solid tumors (n = 17) were also typed for HA-1. The resulting allelic frequencies were very similar to those observed in healthy individuals (Table 2), thus suggesting that HA-1 variants

Table 2. *Allelic Frequencies of HA-1 and HA-2 in Healthy Individuals or Cancer Patients from Italy*

Variable	Allelic Frequency (%)	
	Healthy Individuals*	Cancer Patients†
$HA\text{-}1^H$	29.3	32.8
$HA\text{-}1^R$	70.7	67.2
$HA\text{-}2^V$	83.7	81.7
$HA\text{-}2^M$	16.3	18.3

HA-1 and HA-2 polymorphisms were characterized by genomic PCR sequence-specific priming as described in "Materials and Methods." In case only 1 allele was detected, homozygosity was assumed, and the allele was counted twice for calculation of allelic frequencies.

*n = 208 for HA-1; n = 199 for HA-2.

†n = 99 for HA-1; n = 91 for HA-2.

Table 3. *Allelic Frequencies of HA-1 in Different White Populations*

	Allelic Frequency (%)									
	Southern Europe				Northern Europe and North America					
Variable	Italy* (n = 210)	Spain† (n = 203)	Greece‡ (n = 98)	Mean§	Dutch‖ (n = 100)	Germany¶ (n = 201)	Germany# (n = 65)	Germany** (n = 545)	United States†† (n = 160)	Mean§
HA-1^H	29.3	32.8	29.6	30.6 ± 1.9	44.3	39.0	45.2	61.3	42.0	46.4 ± 8.7
HA-1^R	70.7	67.2	70.4	69.4 ± 1.9	55.7	61.0	54.8	38.7	58.0	53.6 ± 8.7

*This study.
†Reported in reference [24].
‡Reported in reference [22].
§Mean ± SD.
‖Reported in references [10] and [23].
¶Reported in reference [21].
#Reported in reference [25].
**Reported in reference [19].
††North American whites, reported in reference [11].

are not involved in the predisposition to or protection from the development of cancer.

A North-South Frequency Gradient for HA-1 Allelic Variants

The allelic frequencies observed for HA-1 variants in this study were compared with those reported by others in healthy white individuals from Southern or Northern Europe and North America [10,11,19,21-25] (Table 3). It is interesting to note that the frequency of HA-1^H was generally higher in populations from the north (mean ± SD, 46.4% ± 8.7%) as compared with the south (30.6 ± 1.9%), whereas the inverse correlation was evident for HA-1^R (mean ± SD, 53.6% ± 8.7% and 69.4% ± 1.9% in northern and southern countries, respectively; Table 3).

Allelic Frequencies of HA-2 in the Italian Population

In 199 healthy blood donors typed for HA-2 polymorphism, allelic frequencies of 83.7% and 16.3% were observed for HA-2^V and HA-2^M, respectively (Table 2). A total of 91 cancer patients studied for HA-1 polymorphism were also typed for HA-2. Again, no skewing of the polymorphism was associated with the presence of a malignant disorder (Table 2). This finding argues against an involvement of HA-2 variants in the pathogenesis of cancer—a possibility theoretically compatible with the role of this protein in formation of the cytoskeleton [12]. The data obtained here for the Italian population are in line with those previously reported for the Dutch, in which the allelic frequency of HA-2^V and HA-2^M was 77.6% and 22.4%, respectively [23,26].

Clinical Applications of HA-1 and HA-2 Polymorphisms

On the basis of our data, we calculated that the probability for a given Italian patient of carrying HLA-A2 (42.8% [28]) and of being mismatched in the GVL direction with his/her allogeneic HSC donor would be 10.7% for HA-1 and 1.1% for HA-2 (Table 4). These predicted percentages were in line with those actually observed in the series of patients who underwent allogeneic HSCT at our center and who presented the possibility of exploiting GVL targeted against HA-1 or HA-2 in 15.1% (15/99) and 1% (1/91) of the cases, respectively (Table 4). It is important to note that the possibility of using patient-specific HA-1 or HA-2 alleles as markers for the state of HC after transplantation can be predicted to be present in 32.8% and 23.5% of Italian transplantation patients, respectively

Table 4. *Clinical Applications of HA-1 and HA-2 Polymorphisms in Allogeneic HSCT*

	% Application for HA-1 Polymorphism		% Application for HA-2 Polymorphism	
Variable	Predicted	Observed*	Predicted	Observed†
GVL‡	10.7	15.1	1.1	1
HC§	32.8	25.6	23.5	16.2

The values in the column "Predicted" were calculated on the basis of allelic frequencies of HA-1 and HA-2 variants in the Italian population, reported in Table 2. The values in the column "Observed" refer to the actual number of pairs with the relevant clinical application in our series.
*Observed: n = 99 for GVL; n = 82 for HC (only patients with hematologic malignancies were considered for HC).
†Observed: n = 91 for GVL; n = 74 for HC (only patients with hematologic malignancies were considered for HC).
‡GVL: potential for graft-versus-leukemia (GVL) reactivity in HLA-identical donor recipient pairs expressing HLA-A2, present with a phenotypic frequency of 42.8% for HLA-A2 in Italy [28]. Calculations were based on the formula specified in "Materials and Methods."
§HC: possibility of HC detection by tracing of HA-1 or HA-2 variants as markers for reappearance of cells of host origin after allogeneic HSCT. Calculations were based on the formula specified in "Materials and Methods."

(Table 4). This prediction reflected the actual situation in our series of patients who received transplants for hematologic malignancies, in whom this application was possible in 25.6% (21/82) and 16.2% (12/74) for HA-1 and HA-2, respectively. A total of 89% (73/82) and 89.1% (66/74) of these patients lacked a tumor-specific molecular marker. In 28.7% (21/73) and 15.1% (10/66) of patients without a tumor-specific marker, HA-1 and HA-2, respectively, could be used as surrogates for the reappearance of host cells, including leukemic blasts. Overall, in the 66 patients without a molecular tumor marker who were typed for HA-1 and HA-2, a patient-specific allele of 1 or both of these mHags could be used as a surrogate marker for disease recurrence in 36.3% (24/66) of cases (data not shown). One of these patients, patient 20, who received a transplant from an HA-2–disparate haploidentical family donor for acute myeloid leukemia, experienced an initial CR followed by disease relapse. HA-2 typing was retrospectively used for disease monitoring in bone marrow aspirates after HSCT (Figure 1). Disease recurrence could be evidenced by tracing the patient-specific HA-2^M allele at the time of relapse.

The similarity of HA-1 allelic frequencies (Table 3) and of HLA-A2 expression [28] in Italy and in other populations from Southern Europe, such as Spain and Greece, suggests that the predictions made for Italy will also apply to other Mediterranean countries. In contrast, in populations from Northern Europe, such as the Dutch and Germans, as well as in North American whites, the frequency of HA-1^H is higher than in the south (Table 3). In addition, in these populations, HLA-A2 is slightly more frequent than in the south (Germany, 47.8%; North American whites, 48.6%; mean, 48.2% [27]). On the basis of these data, it can be predicted that HA-1 can be exploited for therapeutic (GVL) or diagnostic (HC) purposes in approximately 9.9% and 37.4%, respectively, of patients from the north (data not shown).

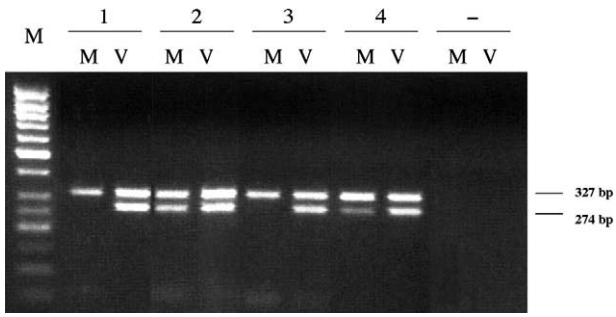

Figure 1. Monitoring of disease recurrence by tracing the patient-specific HA-2^M allele in patient 20. Genomic HA-2 typing of patient 20 and her haploidentical family stem cell donor was performed as described in "Materials and Methods." PCR fragments of 327 and 274 bp correspond to the internal control gene and the HA-2 allele, respectively. *M* and *V* stand for the polymorphism HA-2^M and HA-2^V, respectively. Agarose gel electrophoresis is shown of PCR products obtained from peripheral blood of the donor (lane 1), of the patient before transplantation (lane 2), and of bone marrow aspirates from the patient drawn in complete clinical remission at day +90 (lane 3) or in relapse at day +150 (lane 4) after HSCT. —, PCR mix amplified in the absence of template DNA. The molecular weight marker used is GeneRuler 50bp DNA ladder (MBI Fermentas, Hanover, MD).

DISCUSSION

The mHags HA-1 and HA-2 have raised considerable interest as targets for selective immunotherapy after allogeneic HSCT because they are among the few mHags characterized at the molecular level that are expressed on hematopoietic tissues and some solid tumors, but not on healthy tissues of different origin. The potential applicability of this approach is, however, hampered by the allelic distribution of HA-1 and, more so, of HA-2, which restricts the percentage of patients predicted to benefit from GVL mediated by these 2 mHags to approximately 10% and 1% for HA-1 and HA-2, respectively. Despite the north-south frequency gradient demonstrated here for HA-1 allelic variants, these numbers do not vary substantially between patients from northern and southern countries because the higher frequency of the immunogenic HA-1^H allele in northern populations is compensated for by a higher frequency of HLA-A2 in these populations [28]. For HA-2, no apparent differences in allelic distribution were found in Italy as compared with The Netherlands [23], the only population analyzed for HA-2 variability so far. More populations need to be studied to confirm the apparent absence of frequency differences for HA-2 variants in northern and southern countries.

This study is the first to systematically compare allelic HA-1 and HA-2 frequencies in different populations and to calculate the possibility of exploiting the disparity for these mHags for clinical or diagnostic purposes in patients undergoing allogeneic HSCT. It is interesting to note that we show here that the potential applications of HA-1 and HA-2 disparity for diagnostic purposes, in terms of determination of HC status after transplantation, are far greater than those for therapeutic purposes in terms of GVL activity. This is because exploitation of mHag disparity for diagnostic purposes is independent from the presence of HLA-A2 and from the presence or absence of an immunogenic or nonimmunogenic variant in the patient or in the donor. In this setting, patient-specific HA-1 or HA-2 alleles can be detected by PCR-based sequence-specific priming typing in the peripheral blood or bone marrow of patients who have received transplants. It is likely that the sensitivity of this method would be improved by using nested PCR, as opposed to single-step PCR. Indeed, nested PCR am-

plification of HA-1 variants has recently been demonstrated to detect HA-1 alleles expressed by as few as 1 in 10^5 cells [20].

We have demonstrated that tracing a patient-specific HA-2 variant could be used for detection of disease recurrence after allogeneic HSCT. It should be noted that the reappearance of cells carrying host-specific HA-1 or HA-2 alleles in their genome is most frequently indicative of disease relapse, but it can also be due to graft rejection or autologous marrow reconstitution, especially if nonmyeloablative transplantation conditioning regimens are used. These possibilities can in some cases be discriminated by performing the analysis on subpopulations of cells expressing defined surface expression markers after fluorescence-activated cell sorting. For instance, T lymphocyte–mediated acute graft rejection is associated with a predominance of host cells in the lymphoid $CD3^+$ compartment, whereas relapsing leukemia will be predominantly present in the subpopulation of cells sorted for markers of the initial leukemic phenotype. For correct interpretation of mixed HC based on the reappearance of host-specific mHags, clinicians also need to consider the transplantation regimen, the presence or absence of T-cell depletion, and the individual relapse risk of the patient on the basis of the underlying disease. Another parameter to be taken into account is the time elapsed after transplantation, because mixed HC is indicative of graft rejection in the early phase, whereas it is more frequently associated with relapse in the later phase after transplantation. On the basis of these considerations, it is evident that the reappearance of host mHags can be used to detect different initiating adverse clinical events, including relapse, but cannot by default be interpreted as indication for the presence of minimal residual disease (MRD).

Conversely, new molecular markers suitable for sensitive detection of MRD after allogeneic HSCT for hematologic malignancies are urgently needed to improve the efficacy of this powerful therapeutic tool, because early MRD detection is a prerequisite to drive appropriate clinical maneuvers such as donor lymphocyte infusions or the withdrawal of immune-suppressive drugs. In fact, many hematologic malignancies that require allogeneic HSCT for treatment lack a tumor-specific molecular marker. In our series, 89% of patients did not have such a marker and, thus, could not be monitored for disease relapse by standard molecular methods. Recent reports have shown that detection of increased transcript levels of the Wilms tumor 1 gene (*WT1*) by real-time reverse transcriptase-PCR can be a promising novel approach of universal monitoring of disease recurrence in myeloid leukemias, which in >50% of patients lack an alternative tumor marker [30]. It is interesting to note that although increased transcript levels of *WT1* have been shown to have a good correlation with relapse in patients at diagnosis and after chemotherapy [31], their predictive value for disease recurrence after allogeneic HSCT is subject to debate [32].

The classical approach to monitor the state of HC after allogeneic HSCT relies on the detection of short tandem repeats, which differ in length between patient and donor and can be characterized by capillary gel electrophoresis after amplification by PCR [33]. In addition, in transplantation from female donors into male recipients, host cells can be traced by using cytogenetics or fluorescent in situ hybridization for male-specific genes on the Y chromosome [33]. The advantage of using mHags as markers for determination of the status of HC relies on the fact that this method is cheap and easy to perform without the requirement for sophisticated instrumentation, independently from donor and recipient sex. In addition, when nested PCR is used, mHags can be detected with an approximately 3-log higher sensitivity [20] as compared with short tandem repeats and fluorescent in situ hybridization, which have a detection threshold of approximately 1% to 5% [33]. The biallelic nature of HA-1 and HA-2 polymorphism renders these markers also suitable for quantitative analysis by real-time PCR. On the basis of these considerations, the use of mHags for analysis of the HC status after transplantation may allow for a cheaper and more timely diagnosis of mixed chimerism as compared with classical markers. As a consequence, their diagnostic application for follow-up of allogeneic HSCT may permit an early and therefore more efficient adequate clinical intervention, thereby ultimately improving the therapeutic efficacy of transplantation.

Taken together, the observations from this study suggest that genomic typing for HA-1 and HA-2, which has a low cost and is easy to perform by most laboratories involved in tissue typing for allogeneic HSCT, can be a powerful therapeutic and diagnostic tool for an appreciable number of patients undergoing allogeneic HSCT from a fully HLA-matched family or unrelated donor. We propose that this analysis should be included in tests used for donor selection, especially for patients with hematologic malignancies who lack a tumor-specific molecular marker.

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REFERENCES

1. Spierings E, Wieles B, Goulmy E. Minor histocompatibility antigens—big in tumour therapy. *Trends Immunol*. 2004;25:56-60.
2. Chao NJ. Minors come of age: minor histocompatibility antigens and graft-versus-host disease. *Biol Blood Marrow Transplant*. 2004;10:215-223.
3. Falkenburg JH, van de Corput L, Marijt EW, Willemze R. Minor histocompatibility antigens in human stem cell transplantation. *Exp Hematol*. 2003;31:743-751.
4. Bleakley M, Riddell SR. Molecules and mechanisms of the graft-versus-leukaemia effect. *Nat Rev Cancer*. 2004;4:371-380.
5. Goulmy E. Minor histocompatibility antigens: allo target molecules for tumor-specific immunotherapy. *Cancer J*. 2004;10:1-7.
6. Hambach L, Goulmy E. Immunotherapy of cancer through targeting of minor histocompatibility antigens. *Curr Opin Immunol*. 2005;17:202-210.
7. den Haan JM, Meadows LM, Wang W, et al. The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. *Science*. 1998;279:1054-1057.
8. den Haan JM, Sherman NE, Blokland E, et al. Identification of a graft versus host disease-associated human minor histocompatibility antigen. *Science*. 1995;268:1476-1480.
9. Klein CA, Wilke M, Pool J, et al. The hematopoietic system-specific minor histocompatibility antigen HA-1 shows aberrant expression in epithelial cancer cells. *J Exp Med*. 2002;196:359-368.
10. Wilke M, Pool J, den Haan JM, Goulmy E. Genomic identification of the minor histocompatibility antigen HA-1 locus by allele-specific PCR. *Tissue Antigens*. 1998;52:312-317.
11. Tseng LH, Lin MT, Martin PJ, Pei J, Smith AG, Hansen JA. Definition of the gene encoding the minor histocompatibility antigen HA-1 and typing for HA-1 from genomic DNA. *Tissue Antigens*. 1998;52:305-311.
12. Pierce RA, Field ED, Mutis T, et al. The HA-2 minor histocompatibility antigen is derived from a diallelic gene encoding a novel human class I myosin protein. *J Immunol*. 2001;167:3223-3230.
13. Mutis T, Verdijk R, Schrama E, Esendam B, Brand A, Goulmy E. Feasibility of immunotherapy of relapsed leukemia with ex vivo-generated cytotoxic T lymphocytes specific for hematopoietic system-restricted minor histocompatibility antigens. *Blood*. 1999;93:2336-2341.
14. Mutis T, Gillespie G, Schrama E, Falkenburg JH, Moss P, Goulmy E. Tetrameric HLA class I-minor histocompatibility antigen peptide complexes demonstrate minor histocompatibility antigen-specific cytotoxic T lymphocytes in patients with graft-versus-host disease. *Nat Med*. 1999;5:839-842.
15. Kircher B, Stevanovic S, Urbanek M, et al. Induction of HA-1-specific cytotoxic T-cell clones parallels the therapeutic effect of donor lymphocyte infusion. *Br J Haematol*. 2002;117:935-939.
16. Marijt WA, Heemskerk MH, Kloosterboer FM, et al. Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. *Proc Natl Acad Sci U S A*. 2003;100:2742-2747.
17. Kircher B, Wolf M, Stevanovic S, et al. Hematopoietic lineage-restricted minor histocompatibility antigen HA-1 in graft-versus-leukemia activity after donor lymphocyte infusion. *J Immunother*. 2004;27:156-160.
18. Kloosterboer FM, van Luxemburg-Heijs SA, van Soest RA, et al. Direct cloning of leukemia-reactive T cells from patients treated with donor lymphocyte infusion shows a relative dominance of hematopoiesis-restricted minor histocompatibility antigen HA-1 and HA-2 specific T cells. *Leukemia*. 2004;18:798-808.
19. Brossart P, Spahlinger B, Grunebach F, et al. Induction of minor histocompatibility antigen HA-1-specific cytotoxic T cells for the treatment of leukemia after allogeneic stem cell transplantation. *Blood*. 1999;94:4374-4376.
20. Wieles B, Pool J, Derks R, Burlingham WJ, Goulmy E. Detection of microchimerism by minor histocompatibility antigen HA-1 allele-specific nested polymerase chain reaction. *Biol Blood Marrow Transplant*. 2005;11:345-348.
21. Heinemann FM, Ferencik S, Ottinger HD, Beelen DW, Grosse-Wilde H. Impact of disparity of minor histocompatibility antigens HA-1, CD31, and CD49b in hematopoietic stem cell transplantation of patients with chronic myeloid leukemia with sibling and unrelated donors. *Transplantation*. 2004;77:1103-1106.
22. Kotzampasaki EM, Spyropoulou-Vlachou MS, Kalofoutis C, Vrani V, Kalofoutis A, Stavropoulos-Giokas C. Minor histocompatibility antigen HA-1 and HPA-5 polymorphisms in HLA-identical related bone marrow transplantation. *Transplant Proc*. 2004;36:1735-1738.
23. van Els CA, D'Amaro J, Pool J, et al. Immunogenetics of human minor histocompatibility antigens: their polymorphism and immunodominance. *Immunogenetics*. 1992;35:161-165.
24. Arostegui JI, Gallardo D, Rodriguez-Luaces M, et al. Genomic typing of minor histocompatibility antigen HA-1 by reference strand mediated conformation analysis (RSCA). *Tissue Antigens*. 2000;56:69-76.
25. Harangi M, Kaminski WE, Fleck M, et al. Homozygosity for the 168His variant of the minor histocompatibility antigen HA-1 is associated with reduced risk of primary Sjogren's syndrome. *Eur J Immunol*. 2005;35:305-317.
26. Wilke M, Pool J, Goulmy E. Allele specific PCR for the minor histocompatibility antigen HA-2. *Tissue Antigens*. 2002;59:304-307.
27. Kimura A, Sasazuki T. Eleventh International Histocompatibility Workshop reference protocol for the HLA DNA-typing technique. In: Tsuyi KI, Aizawa M, Sasazuki T, eds. *HLA 1991. Proceedings of Eleventh International Histocompatibility Workshop and Conference*. Oxford, UK: Oxford University Press; 1991:397-419.
28. Clayton J, Lonjou C, Whittle D. Allele and haplotype frequencies for HLA loci in various ethnic groups. In: Charron D, ed. *HLA Genetic diversity of HLA Functional and Medical Implication. Proceedings of the Twelfth International Histocompatibility Workshop and Conference*. Paris, France: EDK; 1997:665-820.
29. Anderton SM, Wraith DC. Selection and fine-tuning of the autoimmune T-cell repertoire. *Nat Rev Immunol*. 2002;2:487-498.
30. Cilloni D, Saglio G. WT1 as a universal marker for minimal residual disease detection and quantification in myeloid leukemias and in myelodysplastic syndrome. *Acta Haematol*. 2004;112:79-84.
31. Weisser M, Kern W, Rauhut S, et al. Prognostic impact of RT-PCR-based quantification of WT1 gene expression during MRD monitoring of acute myeloid leukemia. *Leukemia*. 2005;19:1416-1423.
32. Uzunel M, Ringden O. Poor correlation of kinetics between BCR-ABL and WT1 transcript levels after allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2004;33:47-52.
33. Khan F, Agarwal A, Agrawal S. Significance of chimerism in hematopoietic stem cell transplantation: new variations on an old theme. *Bone Marrow Transplant*. 2004;34:1-12.