The Relationship between Minor Histocompatibility Antigens and Graft Versus Host Disease in Unrelated Peripheral Blood Stem Cell Transplants

Running Title: mHags and GVHD

Conflict of interest disclosure: The author declares no competing interests relevant to this article.

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Abstract

Background: The goal of this study was to determine the relationship between the presence of minor histocompatibility antigens (mHags) and the development of Graft versus Host Disease (GVHD). Due to the long term effects GVHD can have on transplant patients and their potential transfusion requirements, our goal was to assess whether testing for the mHags should be done on a prospective basis. A cohort of 45 patients with hematologic malignancies and their 10 of 10 HLA-matched unrelated donor pairs were retrospectively assessed for the presence of mHags.

Study Design/Methods: The 45 patient/donor pairs in this study were typed for 19 mHags using a PCR-SSP typing kit and gel electrophoresis. GVHD data was extracted from the clinical protocol database. Statistical analysis was performed using the Fisher exact test.

Results: Patients with minor mismatches were analyzed for both acute and chronic GVHD. Preliminary analysis demonstrates that out of the 45 patient/donor pairs, 30 pairs had mHags mismatched in the GVHD direction. Nineteen of the 30 patients with mismatches developed acute GVHD ($p=0.062$) and 15 of the 30 patients with mismatches developed chronic GVHD ($p=0.061$).

Conclusion: The PCR-SSP kit provided useful information to compare patients and their donors, however there was no statistically significant correlation ($p=0.062$) between mHag mismatches and the development of GVHD. This may have been due to the limited sample size. In order to fully determine a possible significance, a larger cohort should be studied to determine the efficacy of testing for the mHags on a prospective basis.

Keywords:

Minor histocompatibility antigens, transplant, Graft versus host disease

Abbreviations:

GVHD	Graft versus Host Disease
HLA	Human Leukocyte Antigen
mHags	Minor histocompatibility antigens
PBSC	Peripheral Blood Stem Cell
PCR	Polymerase chain reaction
PCR-SSP	PCR with sequence specific primers

Introduction

The Human Leukocyte Antigens (HLA) play a major role in transplantation medicine, especially in stem cell transplants. There are two classes of HLA molecules: Class I includes the A, B and C loci and Class II includes the DP, DQ and DR loci. Class I molecules are located on platelets and most nucleated cells in the body, whereas Class II molecules are located on macrophages, B lymphocytes, dendritic cells and monocytes[1]. HLA antigens work with the T cells of the immune system to help distinguish self from non-self. A higher match between patient and donor in regards to the HLA system should help to ensure a more successful transplant. However, Graft versus Host Disease (GVHD) remains a major obstacle in allogeneic stem cell transplants.[2]

GVHD occurs because the donor or "graft" sees the recipient or "host" as foreign and essentially attacks recipient tissues. GVHD is clinically characterized in two states: acute and chronic. Acute GVHD typically occurs in the first 100 days of transplant and chronic GVHD can occur anytime after the first 100 days.[3] Acute GVHD can occur in 15% to 40% of stem cell transplant patients and is a major cause of morbidity, while chronic GVHD can occur in 50% of patients who survive 90 days after transplant.[2] GVHD affects the skin, liver and gut. Typically it can cause blistering rashes, high levels of bilirubin and the liver enzyme aspartate transferase (AST), and diarrhea.[4] Patients are graded based on the severity of these symptoms and are categorized as I-IV, with IV being the most severe. It is thought that one of the mechanisms of GVHD is donor cytotoxic T cells directed against mismatched recipient minor histocompatibility antigens.[5,6]

The term minor histocompatibility antigens (mHags) may be misleading, as mHags are certainly not "minor" when it comes to transplantation medicine. mHags are peptides that are produced by an immunogenic allele variant and are presented to the immune system by HLA Class I and Class II antigens.[7] Even when a patient and donor are a full HLA match, they may have different allele variants of the mHags and produce different peptides. It is these mismatched variants that cause the peptides to be viewed as foreign, potentially resulting in GVHD. The alleles that cause these peptide variants arise due to single nucleotide polymorphisms (SNPs) at the DNA level.[7]

Each mHag can only be presented by a certain type of HLA molecule, which is known as "restriction".[8,9,10] Table 1 lists the mHags that were tested in this study and shows the HLA restriction for each mHag.[11] The mHag genes can be found on different chromosomes, including the Y chromosome. They can be expressed on many different tissues in the body.[11]

Materials and Methods

Study Cohort - Since 2007, matched-unrelated peripheral blood stem cell transplants have been performed at our facility under a protocol that monitors Graft versus Host Disease. From this protocol, 48 patient pairs who were a 10/10 match at the HLA-A, B, C, DR and DQ loci and received a PBSC transplant due to a hematologic malignancy were evaluated. In this retrospective study, data from 45 of the 48 patient pairs were included. Three pairs were excluded from our study for the following reasons: a donor sample from one patient pair had an insufficient sample size to be tested, a patient sample from another patient pair could not be located, and one patient had previously received a sibling-matched transplant and had seroconverted to the previous donor type. Donor characteristics can be seen in Table 2.

mHag Testing - The 45 patient pairs were tested for the minor histocompatibility antigens using a kit (Minor Histocompatibility Antigen Typing Kit; Invitrogen, Brown Deer, WI), which tests for the mHags using PCR-SSP, along with gel electrophoresis to visual the DNA. PCR-SSP uses sequence specific primers that correspond to both the normal and immunogenic alleles of each of the mHags. Frozen DNA from patients and donors was originally extracted from whole blood specimens or peripheral blood lymphocyte samples. The mHag kit tests for 19 mHags: HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, HwA-9, HwA-10, UGT2B17, HY, LB-ECGF-1, HwA-11, CTSH, CD31 Exons 3, 8 and 12, LRH-1 and LB-ADIR. The sequence specific primers are pre-filled in the wells of the kit, and a mixture of DNA, buffer and TAQ polymerase are added to the primers in the wells. A negative control well is also part of the kit to ensure that the buffer and TAQ polymerase are free of contamination.

mHag Mismatch Assessment - Each patient pair was evaluated for relationship between the mHag genotypes and a known HLA restriction. After mHag testing, each donor and patient pair were evaluated for mismatches in the mHags. In this study, we were interested in those

mismatches that could potentially cause GVHD. This can occur when the donor is negative for a minor antigen that the patient expresses.

GVHD evaluation - GVHD determination and grading was made by the clinical team. Each patient was assessed for acute and chronic GVHD. Patients who relapsed or showed residual disease that required an intervention of chemotherapy or donor lymphocyte infusion were considered non-evaluable (N/E) and were no longer evaluated for GVHD. Of the 45 patient pairs that were tested, 4 patients were evaluated for acute GVHD, but were non-evaluable for chronic GVHD. One patient was non-evaluable for both acute and chronic GVHD. In total, 44 out of 45 patients were assessed for acute GVHD and 40 out of 45 patients were assessed for chronic GVHD.

Statistics - Statistical analysis was performed using 2 x 2 contingency tables, along with the Fisher exact test. $p<0.05$ was considered statistically significant.

Results

Out of the 45 patient/donor pairs, 30 pairs had mismatches in the mHags in the GVHD direction. Of these 30 pairs who had mismatches, 19 of these patients developed acute GVHD and 15 of these patients developed chronic GVHD. Nine of these patients developed both acute and chronic GVHD. Four patients who had mismatches in the GVHD direction did not develop either acute or chronic GVHD.

Out of the 45 patient/donor pairs, 15 pairs did not have mismatches in the GVHD direction. Of these 15 pairs who did not have mismatches, 5 of these patients developed acute GVHD and 10 of these patients developed chronic GVHD. Four of these patients developed both acute and chronic GVHD. Two patients who did not have mismatches in the GVHD direction did not develop either acute or chronic GVHD.

Using 2 x 2 contingency tables and the Fisher exact test that accounted for those patients with and without mismatches and those who were positive and negative for acute GVHD, the statistical significance for acute GVHD was $p= 0.062$. The same table and testing was used for

those patients who were positive and negative for chronic GVHD and the statistical significance for chronic GVHD was p= 0.061. The contingency tables can be seen in Table 3.

The 30 patients who had mismatches in the mHags in the GVHD direction were evaluated to see if the number of mismatches had any correlation with the occurrence of either acute or chronic GVHD. The number of mismatches ranged from 0 to 6. As shown in Figure 1, the patient with 6 mHag mismatches did not develop either acute or chronic GVHD, while patients who had less mHag mismatches developed some form of GVHD. It does not appear that any correlation can be made between the number of mHag mismatches and the development of GVHD within this small cohort of patients.

Each mHag was evaluated separately for the incidence of acute or chronic GVHD it may have caused in the patients who had a mismatch with their donor. Eleven of the 19 mHags tested were found in both acute and chronic forms of GVHD. mHag ACC-2 was only found in chronic GVHD, while mHag UGT2B17 was only found in acute GVHD. mHag HA-2 was not found in any cases of GVHD. However, because the overall sample size was so small, a definitive correlation cannot be determined.

Discussion

The role the mHags play in transplantation medicine is unknown. However, it is well established that the mHags can only be presented by certain HLA molecules. For example, the mHag HA-2 can only be presented by an HLA-A*02 molecule.[11] A patient and donor would both have to be an HLA-A*02 for the mHag HA-2 to be a concern. It is also known that patients and donors would need to differ in the presence of the immunogenic allele in order for the mHags to be viewed as foreign by the patient's immune system. mHags have been shown to be present on certain tissues of the body, but the scope of this distribution is not completely known. Some data suggests that mHags with broad tissue distribution may be more relevant in GVHD.[12] The PCR-SSP kit used in this study currently tests for 19 of the known mHags, but further research may show that there are even more mHags. If discovery proves that there are more mHags, their relevance, immunogenic alleles, HLA restriction and tissue distribution will need investigation.

As seen in this study, it appears that the mHags may play a role in the development of GVHD. Out of the 30 patients who had mismatches in the mHags in the GVHD direction with their donor, 25 patients developed some form of GVHD, whether it was acute, chronic or both. The data suggests a trend regarding the relevance for mismatching of mHags and the development of GVHD. However, this study was limited to 45 patient pairs, only 30 of whom had mismatches in the mHags in the GVHD direction. A larger cohort in a future study will be needed to determine if there is a statistically significant correlation.

If a significant correlation can be determined, it may be beneficial for patients to be evaluated on a prospective basis for mismatches in the mHags. Testing on a prospective basis could give clinicians more knowledge to help assess the risk of their transplant patients developing GVHD. Personalized medicine is more commonplace, and a patient's mHag profile may help to determine treatment options. If multiple donors can be found for a patient, those with a full HLA match including the mHags may be more beneficial. Patient conditioning could also be adjusted so that patients with donors who have mismatches in the mHags could undergo more aggressive preparative regimens.[13] Perhaps different treatments before transplant may be beneficial in preventing or lessening the effects of GVHD. It could also be argued that if a patient has a donor with a full HLA match, including the mHags, perhaps their treatment before transplant could be less debilitating.

As stem cell transplant use widens, continuing insight into the mHags will be beneficial for patients, their physicians and their families. As well as mortality, GVHD can cause lingering detrimental effects on patients including physical pain and suffering.[2] Additional research that can alleviate this complication from transplant from the patients and from healthcare as a whole is urgently needed.

References

1. Roback JD, Grossman BJ, Harris T, Hillyer CD. The HLA System, AABB Technical Manual 17th Edition, 2011, p 547-566
2. Sun Y, Tawara I, Toubai T, Reddy P. Pathophysiology of Acute Graft-vs-Host Disease: Recent Advances, *Transl Res*. 2007 October; 150(4): p 197-214
3. Harmening D, Transfusion Therapy and Transfusion in Transplantation, Modern Blood Banking & Transfusion Practices 5th Edition, 2005, p 311
4. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED. Consensus conference on acute GVHD grading, *Bone Marrow Transplant* 1995 Jun; 15(6): p 825-828
5. Larsen ME, Kornblit B, Larsen MV, Masmas TN, Nielsen M, Thiim M, Garred P, Stryhn A, Lund O, Buus S, Vindelov L. Degree of Predicted Minor Histocompatibility Antigen Mismatch Correlates with Poorer Clinical Outcomes in Nonmyeloablative Allogeneic Hematopoietic Cell Transplantation, *Biol Blood Marrow Transplant* 2010 Oct; 16(10): p 1370-81
6. Edinger M, Powrie F, Chakraverty R. Regulatory Mechanisms in Graft-versus-Host Responses, *Biol Blood Marrow Transplant* 2009 Jan; 15(1): p 2-6
7. Spellman S, Warden MB, Haagenson M, Pietz BC, Goulmy, E, Warren EH, Wang T, Ellis TM. Effects of mismatching for Minor Histocompatibility Antigens on clinical outcomes in HLA-matched, unrelated hematopoietic stem cell transplants, *Biol Blood Marrow Transplant* 2009 July; 15(7): p 856-863
8. Markiewicz M, Siekiera U, Karolczyk A, Szymszal J, Helbig G, Wojnar J, Dzierzak-Mietla M, Kyrcz-Krzemien S. Immunogenic disparities of 11 minor histocompatibility antigens (mHAs) in HLA-matched unrelated allogeneic hematopoietic SCT, *Bone Marrow Transplantation* 2009 Feb; 43(4): p 293-300
9. Perreault C, Decary F, Brochu S, Gyger M, Belanger R, Roy D. Minor histocompatibility antigens, *Blood* 1990 Oct 1; 76(7): p 1269-80
10. Simpson E, Roopenian D, Goulmy E. Much ado about minor histocompatibility antigens, *Immunol Today* 1998 Mar; 19(3): p 108-112
11. Leiden University Medical Center, *The minor Histocompatibility Knowledge Database* 2006 http://www.lumc.nl/rep/cod/redirect/5033/dbminor/index.asp?page=home.html
12. Hambach L, Spierings E, Goulmy E. Risk assessment in haematopoietic stem cell transplantation: Minor histocompatibility antigens, *Best Practice & Research Clinical Haematology* 2007; 20(2): p 171-187
13. Grumet FC, Hiraki DD, Brown BW, Zehunder JL, Zacks ES, Draksharapu A, Parnes J, Negrin R. CD31 Mismatching Affects Marrow Transplantation Outcome, *Biol Blood Marrow Transplant* 2001; 7(9): p 503-

Table 1: mHag Alleles and HLA Restriction

Minor Antigen	Polymorphism*	HLA Restriction	Minor Antigen	Polymorphism*	HLA Restriction
HA-1	H R	HLA-A*02, HLA-B*60	LRH-1	5C 4C	HLA-B*07
HA-2	V M	HLA-A*02	LB-ECGF-1	H R	HLA-B*07
HA-3	T M	HLA-A*01	CTSH	R G	HLA-A*31, HLA-A*33
HA-8	R P	HLA-A*02	LB-ADIR	F S	HLA-A*02
HB-1	H Y	HLA-B*44	HY	HY	HLA-A*01, HLA-A*02, HLA-A*33, HLA-B*07, HLA-B*08, HLA-B*52, HLA-B*60, HLA-DRB1*15:01, HLA-DRB3*03:01, HLA-DQB1*05 (except 05:03 and 05:04)
ACC-1	Y C	HLA-A*24	HwA-11	S T	HLA-A*0201
ACC-2	D G	HLA-B*44	CD31 Exon 3	80V-125V 80V-125L	HLA-A*02
HwA-9 (SP110)	R G	HLA-A*03		80M-125V 80M-125L	
HwA-10 (PANE1)	R Stop	HLA-A*03	CD31 Exon 8	N S	HLA-B*37, HLA-B*41, HLA-B*44, HLA-B*45, HLA-B*47, HLA-B*49, HLA-B*50, HLA-B*60, HLA-B*61
UGT2B17	Ex1a Gene deletion	HLA-A*29, HLA-B*44	CD31 Exon 12	R G	HLA-B*37, HLA-B*41, HLA-B*44, HLA-B*45, HLA-B*47, HLA-B*49, HLA-B*50, HLA-B*60, HLA-B*61

*** Red color indicates immunogenic allele**

Table 2: Patient/Donor Pair Characteristics

Characteristic	n	Mean or %
Patients		
Male	28	62%
Female	17	38%
Total	45	100%
Age*		
Patient at Transplant	21 - 69	48.5
Donor	19 - 58	35
Difference between Donor and Patient	0 - 41	17.8
Age differences*		
Younger Patient/Older Donor	9	21%
Older Patient/Younger Donor	33	77%
Same Age	1	2%
Gender Match between Donor and Recipient**		
Male/Male	24	55%
Female/Female	5	11%
Male/Female	4	9%
Female/Male	11	25%
ABO Incompatibility		
None	25	55%
Major	8	18%
Minor	9	20%
Major and Minor	3	7%
Rh Incompatibility		
Yes	12	27%
No	33	73%
CMV Status		
Positive/Positive	19	42%
Negative/Negative	14	31%
Positive/Negative	7	16%
Negative/Positive	5	11%

* This information was not available for 2 pairs.

** This information was not available for 1 pair.

Table 3: 2 x 2 Contingency Tables for Acute and Chronic GVHD

		Acute GVHD Pos	Acute GVHD Neg	
mHags mismatches in GVHD direction	Pos	19	11	30
	Neg	5	9	14
		24	20	

Fisher exact test p = 0.0621

		Chronic GVHD Pos	Chronic GVHD Neg	
mHags mismatches in GVHD direction	Pos	15	13	28
	Neg	10	2	12
		25	15	

Fisher exact test p=0.0614

Note : one patient unable to be evaluated for Acute and Chronic GVHD, 4 patients unable to be evaluated for Chronic GVHD

Figure 1: Incidence of GVHD compared to mHag mismatches. The data is presented to compare the number of mismatches in the mHags between patients and their donors with the occurrence of either acute or chronic GVHD. N/E = not evaluable for GVHD.