

Expanding the immunotherapeutic potential of minor histocompatibility antigens

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Commentary

Minor histocompatibility antigens (mHAgs) selectively expressed by cells or cell subsets of the hematopoietic system are targets of the T cell–mediated graft-versus-leukemia response that develops following allogeneic hematopoietic stem cell transplantation (HSCT) for the treatment of hematological malignancies. This observation has served as the rationale for utilizing mHAg-specific immunotherapy for the treatment of particular patients. However, at present, only a select and small number of patients could potentially benefit from mHAg-based immunotherapy. A report from de Rijke et al. in this issue of the *JCI* describes a new hematopoietic lineage–specific HLA-B7–restricted mHAg associated with remission of chronic myeloid leukemia. This result represents another example of an mHAg-mediated graft-versus-leukemia response, thereby expanding the number of patients eligible for mHAg-based immunotherapy in the setting of HSCT.

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Expanding the immunotherapeutic potential of minor histocompatibility antigens

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Minor histocompatibility antigens (mHAg) selectively expressed by cells or cell subsets of the hematopoietic system are targets of the T cell-mediated graft-versus-leukemia response that develops following allogeneic hematopoietic stem cell transplantation (HSCT) for the treatment of hematological malignancies. This observation has served as the rationale for utilizing mHAg-specific immunotherapy for the treatment of particular patients. However, at present, only a select and small number of patients could potentially benefit from mHAg-based immunotherapy. A report from de Rijke et al. in this issue of the JCI describes a new hematopoietic lineage-specific HLA-B7-restricted mHAg associated with remission of chronic myeloid leukemia (see the related article beginning on page 3506). This result represents another example of an mHAg-mediated graft-versus-leukemia response, thereby expanding the number of patients eligible for mHAg-based immunotherapy in the setting of HSCT.

Nonstandard abbreviations used: GVHD, graft-versus-host disease; GVL, graft-versus-leukemia; HSCT, hematopoietic stem cell transplantation; LRH-1, lymphoid-restricted histocompatibility antigen-1; mHAg, minor histocompatibility antigen.

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Characteristics of minor histocompatibility antigens applicable for immunotherapy

Minor histocompatibility antigens (mHAg) were originally defined in mice by characterization of in vivo rejection responses to skin grafts and tumors exchanged between mice of different inbred strains (1, 2). Simultane-

ous with the discovery that matching HLA antigens are necessary for optimal success of allogeneic BM transplantation (3), clinical results demonstrated the powerful alloimmune reactions against mHAg. In an HLA-matched hematopoietic stem cell transplantation (HSCT) setting, mHAg disparities between recipient and donor can lead to graft-versus-host disease (GVHD) (4) or graft rejection (5). Aside from these detrimental effects, the mHAg-induced alloimmune response also causes the curative graft-versus-leukemia (GVL) effect. Since mHAg-specific T cells are involved in both GVHD and GVL, dissecting the role of these cells in the immunobiology of GVHD and GVL has proven challenging. The first indication that led us to propose the use of mHAg as immunotherapeutic tools in HSCT (6) was provided by the results of in vitro studies showing differential modes of recognition of various cell types by mHAg-specific CTLs, i.e., ubiquitous or hemato-



Table 1
Immunotherapeutic mHAGs

mHAG	mHAG disparity ^A	HLA-restriction molecule	HLA frequency ^B	Overall applicability	mHAG gene	mHAG tissue distribution	Reference
HA-1	24%	HLA-A2	43%	10.6%	<i>HA-1</i>	Hematopoietic cells, myeloid and lymphoid leukemic cells	(29)
HA-2	4%	HLA-A2	43%	1.7%	<i>Myosin 1G</i>	Hematopoietic cells, myeloid and lymphoid leukemic cells	(15)
HB-1 ^{H/Y C}	6%/24%	HLA-B44	12%	0.7%/2.9%	Unknown	B cell ALL	(16)
ACC-1	17%	HLA-A24	34%	5.8%	<i>BCL2A1</i>	Hematopoietic cells, myeloid and lymphoid leukemic cells	(27)
ACC-2	17%	HLA-B44	12%	2.7%	<i>BCL2A1</i>	Hematopoietic cells, myeloid and lymphoid leukemic cells	(27)
UGT2B17	11%	HLA-A29	5%	0.6%	<i>UGT2B17</i>	DCs, B cells	(17)
LRH-1	13% ^D	HLA-B7	11%	1.4%	<i>P2X5</i>	T cells, B cells, NK cells, myeloid leukemic progenitor cells	(28)
B8/H-Y	25%	HLA-B8	8%	2.0%	<i>UTY</i>	Hematopoietic cells	(30)

^ADisparity within the transplant pairs with the correct HLA allele. Calculations were based on the reported allele frequencies under the assumption of an HLA-matched unrelated donor. ^BPhenotype frequencies were calculated based on global allele frequencies reported in dbMHC (<http://www.ncbi.nlm.nih.gov/mhc>). ^CHB-1 can be recognized bidirectionally. Data represent, respectively, the disparity for HB-1^H and HB-1^Y as positive alleles. ^DIt has not been reported whether the disparity rate for LRH-1 was determined in HLA-matched sibling pairs or in HLA-matched unrelated donor/recipient pairs. ALL, acute lymphocytic leukemia.

poietic system-restricted (7). mHAGs are ubiquitously expressed, including on fibroblasts, melanocytes, and keratinocytes; cell types present within organs affected by GVHD. The CTLs directed to the ubiquitous mHAGs are therefore particularly relevant to the development of GVHD. In addition to in vitro cellular analyses, an in situ readout was performed to analyze the postulated differential in vivo effects of mHAGs. For this, an ex vivo in situ skin explant assay was used, wherein skin sections were incubated with CTLs specific for a ubiquitously expressed mHAG, H-Y, or for the hematopoietic system-restricted mHAGs HA-1 and HA-2 (8). CTLs specific for the H-Y mHAG induced severe graft-versus-host reactions of grades III-IV. CTLs specific for HA-1 and HA-2 induced no or weak graft-versus-host reactions.

mHAGs with tissue expression limited to cells of the hematopoietic system are especially relevant to GVL activity. CTLs specific for hematopoietic system-restricted mHAGs are capable of lysing leukemic cells in vitro (9) and in vivo in a translational mouse model (E. Goulmy et al., unpublished observations). In the clinical setting of HSCT, complete hematological responses and conversion from mixed to complete donor chimerism after donor lymphocyte infusion (DLI) for the treatment of chronic myeloid leukemia and multiple myeloma are associated with a rapid increase in the numbers of functional HA-1- and HA-2-specific T cells in peripheral blood (10).

These data strongly suggest that donor T cells specific for hematopoietic system-restricted mHAGs expressed on recipient cells can be involved in the induction and/or maintenance of remission of hematological malignancies after HSCT.

The accumulated in vitro and in vivo data underline the proposition that mHAGs could be used to induce the curative effect of HSCT. It is noteworthy that this application is not restricted to hematological malignancies but extends to solid tumors as well (11). Protocols have been established for the in vitro generation of donor-derived HA-1- or HA-2-specific CTLs to treat recurrence of the original disease after HLA-matched HA-1-mismatched and/or HLA-matched HA-2-mismatched HSCT (12). A potentially efficient strategy is vaccination of patients by boosting the donor GVL response at appropriate times after HSCT with minor histocompatibility peptides. Currently, an HA-1/HA-2 phase I/II vaccination trial for HLA-A2/HA-1-positive and/or HLA-A2/HA-2-positive patients with advanced hematological malignancies receiving HLA-matched HA-1-mismatched and/or HLA-matched HA-2-mismatched HSCT is ongoing. It is hoped that this approach will elicit allogeneic responses against mHAGs HA-1 or HA-2 and will result in an anti-leukemic effect (Koen van Besien, University of Chicago, Chicago, Illinois, USA, personal communication). We expect that the results of this trial will serve as proof-of-principle

and will lay the basis for second-generation vaccination trials.

Current possibilities for mHAG-specific immunotherapy

The immunotherapeutic potential of cell- and/or tissue-restricted mHAGs demands serious searches for new mHAGs. Information on their phenotypic frequency, tissue distribution, functional membrane expression, and epidemiology is indispensable. The disparity rate of the mHAG between 2 unrelated individuals combined with the allele frequency of the HLA restriction molecule determines its overall applicability. With an overall applicability rate of 10.6%, HA-1 is currently the most interesting candidate for mHAG-based immunotherapy. So far, only 6 other mHAGs with hematopoietic system-specific tissue distribution have been described; 5 are encoded by autosomal genes, and 1 is encoded on the Y chromosome (Table 1; reviewed in ref. 13). Despite inclusion of these mHAGs, the potential number of patients that could be treated remains low due to the phenotypic frequencies of the mHAGs and the HLA restriction molecule.

mHAG identification systems

Various biochemical and molecular approaches have been used to characterize mHAGs. The classical way to identify human mHAGs is elution of peptides from the relevant HLA molecules. The strength of this approach is that the identified



peptide is by definition present on the cell surface (14, 15). The recent determination of the complete human genome sequence has facilitated identification of the gene encoding the relevant peptide. The drawback of the classical approach is the need for highly specialized equipment and personnel. Alternatively, cDNA library screening has successfully been executed for the identification of antigenic minor histocompatibility peptides. Although this technique can be applied for identifying autosomal mHAgS (16, 17), it is particularly powerful for identifying H-Y epitopes, for which there are only a limited number of candidate genes (18–21).

Another possibility for the chemical identification of human mHAgS was put forward by Gubarev et al. in 1996 (22); these authors suggested application of genetic linkage analysis to identify mHAg loci. The method uses EBV-transformed lymphoblastoid cell lines from large families (for example, from the Centre d'Etude du Polymorphisme Humain panel). The families studied consist of 3 generations, and all individuals have been typed for 3,000–10,000 genetic markers (23, 24). This approach led to the localization of mHAgS on chromosomes 22 (22) and 11 (25). At that time, these mHAg loci could not be further refined, leaving the biochemical structure of the epitopes unsolved. The first indications that this approach could indeed lead to the molecular identification of minor histocompatibility peptides were provided by a retrospective study on the HA-8 antigen (26). Combining the genetic linkage data with HLA-binding prediction tools on nonsynonymous single nucleotide polymorphism-containing DNA sequences yielded an epitope that matched the eluted one. Subsequently, this methodology was utilized for the molecular identification of 2 *BCL2A1*-encoded mHAg T cell epitopes, i.e. ACC-1 and ACC-2 (27). The genetic linkage analyses combined with the T cell reactivities specific for mHAgS in question resulted in 46 candidate genes. Further identification was facilitated by the fact that ACC-1- and ACC-2-specific T cell clones only recognize cells of the hematopoietic system. The only gene that was reported by databases to match the expression pattern was *BCL2A1*. Peptide reconstitution assays finally resolved the biochemical identity of the ACC-1 and ACC-2 mHAg T cell epitopes.

In this issue of the *JCI*, de Rijke et al. describe an identical approach that they used in order to identify lymphoid-restrict-

ed histocompatibility antigen-1 (LRH-1) (28). To circumvent the problem that tissue distribution data in the various databases might be incomplete or incorrect, real-time PCR analysis of candidate genes was performed. This additional selection procedure appeared to be crucial for identifying the correct gene. The results clearly show that molecular identification via genetic linkage analyses can successfully be executed for mHAgS with a limited tissue distribution. Genetic linkage identification of minor histocompatibility epitopes with a broad expression pattern, such as the GVHD-associated mHAgS, might turn out to be more difficult. For the identification of leukemia-specific mHAgS and mHAgS that are not expressed by EBV-transformed lymphoblastoid cell lines, this approach is not applicable.

Implications of LRH-1 use for adoptive immunotherapy

The novel mHAg LRH-1 is encoded by the hematopoietic system-specific *P2X5* gene, which has interesting properties with respect to HSCT-based immunotherapy of hematological malignancies. First, *P2X5* transcripts were only detected in lymphoid cells and myeloid leukemia progenitor cells. De Rijke et al. analyzed the presence of LRH-1-specific T cells following HSCT and DLI in a patient with chronic myeloid leukemia (28). A massive rise in the number of LRH-1-specific CTLs coincided with a reduction in the number of Bcr-Abl-positive cells, indicating a potential role for these T cells in the clinical response to LRH-1-expressing CD34⁺ leukemia progenitor cells.

In addition to their hematopoietic system-restricted expression, the mHAg phenotypic frequency and frequency of its HLA restriction molecule represent significant characteristics of mHAgS that make them suitable for use in adoptive immunotherapy. Within the transplant pairs with the correct HLA allele, de Rijke et al. report a 13% disparity, a situation where the transplant donor is negative, and the transplant recipient is positive, for the LHR-1 antigen (28). The LRH-1 mHAg is presented to the immune system by the HLA-B7 molecule. With a phenotype frequency in the range of 10–25%, HLA-B7 is among one of the more frequent HLA alleles (according to a search of the dbMHC; <http://www.ncbi.nlm.nih.gov/mhc>). The combined LHR-1 and HLA-B7 phenotypic frequency clearly positions LHR-1 on the list of candidate mHAgS

suitable for immunotherapy of hematological malignancies. More importantly, the applicability of *P2X5* gene products might exceed that of the LRH-1 epitope. De Rijke et al. found that the *P2X5* gene of the HSCT recipient contained a deletion of a single nucleotide, resulting in a frameshift. To our knowledge, generation of an mHAg via nucleotide deletion/insertion has not been described before and presents interesting opportunities to further exploit the *P2X5* gene product as a source for mHAgS to be used for immunotherapy. HSCT donor T cells might be able to recognize peptides derived from the recipient's *P2X5* gene product fragment following the frameshift. Further investigations using the reverse immunology strategy on this part of the protein might yield new mHAgS in the context of the frequent HLA class I alleles. Moreover, it would be of interest to evaluate whether the C-terminal part of the *P2X5* gene product might contain HLA class II-restricted mHAgS. Evidently, CD4 T cell help during the *in vitro* and/or *in vivo* generation of LRH-1-specific CTLs is crucial. Identification of a combination of functionally different types of mHAgS will definitely further support successful mHAg-based immunotherapy.

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Flushing out the role of GPR109A (HM74A) in the clinical efficacy of nicotinic acid

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The recent discovery of the G_i protein–coupled receptor GPR109A (HM74A in humans; PUMA-G in mice) as a receptor for nicotinic acid has provided the opportunity to gain greater understanding of the underlying biology contributing to the clinical efficacy (increases in HDL, decreases in VLDL, LDL, and triglycerides) and the characteristic side-effect profile of nicotinic acid. GPR109A has been proven to be the molecular target for the actions of nicotinic acid on adipose tissue, and in this issue of the *JCI*, Benyó et al. have confirmed the involvement of GPR109A in the nicotinic acid–induced flushing response, a common side effect (see the related article beginning on page 3634). The involvement of GPR109A in both the desirable and undesirable clinical actions of nicotinic acid raises interesting questions regarding the function of this receptor.

The observation that nicotinic acid can modify lipoprotein profiles in humans was first made in the 1950s. Subsequent clinical experience has demonstrated that nicotinic acid produces a very beneficial modification of multiple cardiovascular risk factors. As a

monotherapy, nicotinic acid is still the most effective therapy for elevating HDL levels while decreasing VLDL and LDL levels as well as other cardiovascular risk factors, which results in a reduction in mortality (1) (Figure 1). In addition to its highly desirable profile of cardiovascular risk factor modulation, nicotinic acid has been shown to produce enhanced therapeutic effects when given in combination with other lipid-lowering drugs (e.g., statins and bile acid resins) (2–3). The past 50 years of nicotinic acid usage has been recently reviewed by Carlson (4).

Identification and function of G_i protein–coupled receptors for nicotinic acid

In 2003, several groups published studies showing that the orphan receptor GPR109A is activated by nicotinic acid at concentrations consistent with the exposure achieved following therapeutic doses (5–7). Furthermore, additional compounds with a clinical profile similar to that of nicotinic acid (e.g., acipimox and acifran) were also confirmed as full agonists of GPR109A. Importantly, nicotinamide, which does not alter lipoprotein profiles but shares the vitamin-like properties of nicotinic acid, has virtually no GPR109A agonist activity. This pharmacological profile strongly suggests that GPR109A is a molecular target involved in the clinical efficacy of nicotinic acid and therefore offers a potential focus to explore the biological processes involved in the highly desirable therapeutic profile achieved following chronic treatment with this drug (8–9).

The best-described action of nicotinic acid is the inhibition of adipocyte lipolysis.

Nonstandard abbreviations used: PGD₂, prostaglandin D₂; PGE₂, prostaglandin E₂; PUMA-G, protein upregulated in macrophages by IFN- γ .

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