Human minor histocompatibility antigens

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Disparities in minor histocompatibility antigens between HLA-matched organ and bone marrow donors and recipients create a potential risk for graft failure and graft-versus-host disease. These conditions necessitate lifelong pharmacological immunosuppression of organ and bone marrow transplant recipients. Recent technical advances have resulted in the identification of the chemical nature of the first human minor histocompatibility antigens. A new era of research has begun to provide insights into the genetics of minor antigens and their putative role in transplantation.

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Abbreviations

BMT	bone marrow transplantation
CTL	cytotoxic T lymphocyte
GVHD	graft-versus-host disease
LCL	lymphoblastoid cell line
mHag	minor histocompatibility antigen
TH	T helper

Introduction

Minor histocompatibility antigens (mHag) have been reported to account for complex events resulting from organ and tissue exchange between MHC identical individuals. The classical definition of mHag dates from 1948 and originates from studies by Snell [1], who described genetic loci responsible for tumor graft rejection. In contrast to major antigens, mHags account for a comparatively slower and more chronic graft rejection [2]. Not having access to the experimental possibilities afforded by inbred and congenic strains, we will never be able to verify the latter statement in man. Yet, in man, simultaneously with the matching for the HLA antigens to improve the success of allogeneic bone marrow transplantation [3], non-HLA (i.e. mHag) became evident.

The possible involvement of mHags in human transplantation was first reported two decades ago [4]. It dealt with a clinical observation in a female patient who received, after ATG pre-treatment, the bone marrow of a male HLA-identical sibling. *In vitro* analysis of the post-transplant peripheral blood lymphocytes of the female patient showed unambiguously that there were strong cytotoxic T lymphocyte (CTL) responses that were specific for the male donor HLA-matched target cells [4,5]. Since this observation, we and others have also described the recognition of human mHags under similar circumstances [6].

Until recently, human mHags were defined by *in vitro* studies of the T-cell reactivity of cells derived from individuals primed *in vivo*. The ability of T cells to mount a response specific for mHags can be described as 'peptide alloreactivity' (i.e. MHC-restricted T cells react with immunogenic peptides). The situation arises between HLA-identical individuals when a mHag is present in one individual, but absent from the other. Isolation and characterization of the human mHag peptides that are reactive in allograft situations has now begun. It is therefore timely to study the genetics and immunogenic potential of human mHags. This review summarizes the recent advances in human mHag immunobiology and discusses these data in relation to its relative clinical importance.

T-cell defined human mHags: genetics and polymorphism

mHags have been defined by MHC-restricted T cells obtained from individuals primed *in vivo* through organ or bone marrow grafting and blood transfusions [6]. Series of mHag-specific MHC class I-restricted CTL populations and MHC class II-restricted T-helper (Th) cells have been described [6,7]. Yet, little information is currently available about population frequencies, segregation patterns and allelism of mHags. From the data gathered so far (see Tables 1 and 2), one may conclude that mHag are recognized in the context of class I and class II alleles, that the majority of T-cell defined mHags are determined by autosomal genes inherited in a Mendelian fashion and that they show either high or low phenotype frequencies. From genetic analysis of our CTL-defined HLA-A2.1-restricted mHag, HA-1, HA-2, HA-4 and HA-5, it was clear that these four antigens can each be considered the product of a gene with one allele expressing the detected specificity, and one or more alleles not expressing it. Although our family data did not provide sufficient information concerning linkage between the different mH loci themselves and HLA, all of our tests were compatible with the hypothesis that these loci are independent of each other and independent of HLA [8].

Unlike the genes encoding mHags in the mouse (as reviewed in [9]), the human genes encoding mHags — with the exception of the gene encoding the male-specific mHag H-Y (see below) — have not yet been identified. It is not yet known how many alleles exist for each locus, nor what the total number of mH loci is. In view of what is known about murine mHags, however, [10,11] a few allelic forms may exist for each locus. The supposedly

low polymorphism may result from the presentation of homologous, but not identical, peptides from the same protein, or failure to present a peptide because it lost an (anchor) amino acid residue. Alternatively, polymorphism in the antigen-processing systems may result in a failure to express the peptide.

Table 1

Cellularly defined class I-restricted mHag.

Restriction molecules	Code (local)	Population frequency	Mode of segregation	Reference
A1	HA-3	88%	Mendelian‡	[23]
A1	H-Y	Males	Y-linked	[65]
A2.1	H-Y	Males	Y-linked	[4]
A2.1	HA-1	69%	Mendelian	[23]
A2.1	HA-2	95%	Mendelian	[23]
A2.1	HA-4	16%	Mendelian	[23]
A2.1	HA-5	7%	Mendelian	[23]
B7	W1	110/141*	Mendelian	[66]
B7	H-Y	Males	Y-linked	[67]
B7	HA-6	15/16	NT	
B7	HA-7	13/15	NT	
B7/B27/B40	–	7/9	NT	[68]
B35	NH5.2†	23/23	Mendelian	[69]
B38	NH54	3/4	Mendelian	[70]
B44	Sol	29/51	Mendelian	[71]
B60	H-Y	Males	Y-linked	[72]

*Number of individuals/total number of individuals tested. †More clones with identical specificity. ‡Mendelian indicates family studies performed. In case of [23], Mendelian traits of inheritance were demonstrated. NT, not tested.

Immunodominant mHag: are there major minors?

mHags are naturally processed peptide fragments originating from intracellular proteins that associate with MHC products. This implies the potential existence of a large number of mHags. The fact that a significant number of bone marrow transplants between HLA-identical sibling (with optimal immunosuppression) do not lead to graft-versus-host disease (GVHD) [12], suggests a hierarchy in immunogenicity. Indeed, *in vitro* studies have detected multiple differences in the mHags between HLA-identical recipient/bone marrow donor combinations [13]. Yet, probably only a limited number of these mHags exhibits immunizing potential, as exemplified by our recent study where we observed a significant correlation between mismatch for one mHag (HA-1) and GVHD in adult patients [14••]. Multiple factors will determine the immunogenic potential of a mHag to activate a T cell. The synergistic effects of mH-specific Th–CTL collaboration, as has been reported for murine mHag [15], may be one such factor.

The peptide affinity for the MHC molecules could also influence the immunological outcome. Using an equilibrium binding assay to measure relative affinities, binding sites of the recently identified mHag HA-2 peptide [16••] classified this peptide among the highest affinity naturally processed peptides that have been identified to date. The concentration of the HA-2 peptide as competitor peptide that resulted in 50% inhibition of the iodinated peptide binding (IC50) was 6.7 nM. IC50% values for other peptides vary from 11–214 nM [17,18]. The sequence of HA-2 included amino acids at positions 4 and 9 that had previously been characterized as strong anchor residues for HLA-A2.1 [19]. The presence of isoleucine at position 2, known to fit the HLA-A2.1 motif, but not recognized as a frequent anchor residue, is of interest. The affinity of MHC class I–peptide binding is crucial for the outcome of an immune response, even in the situation of subdominant epitopes [20•]. Nevertheless, as pointed out by Barber and Parham [21], a number of factors other than peptide MHC affinity determine the successful outcome of an immune response.

Table 2

Cellularly defined class II-restricted mHag.

Restriction molecules	Code (local)	Population frequency	Mode of segregation	Reference
DR2/DR3	LG2	13/20	Mendelian†	[23]
DR2	PN2	17%	Mendelian	[23]
DR2	ID10	10/25	Mendelian	[73]
DR3	H-Y	Males	Y-linked	[73]
DR5	Bur-1	40%	Mendelian	[74]
DRw6/DRw10	LH3	11/24	NT	[23]
DR9	A2*	5/20	NT	[75]
DP4	LG29	NT	Mendelian	[a]
DR2	11	NT	NT	[76]
DR/DP/DQ	–	NT	NT	[77]

[a] E Goulmy, unpublished data. *More clones were obtained with identical specificity. †Mendelian indicates that family studies were performed. In [23], Mendelian traits of inheritance were demonstrated. NT, not tested.

Hierarchy in immunodominance amongst mHags may be reflected in a skewed T-cell repertoire usage for mHag-specific responses. In our study, we observed a limited TCR Vβ region usage, as well as remarkable similarities within the N-D-N regions among mHag HA-1-specific CTL clones derived from unrelated individuals [22•]. Moreover, CTL clones reactive for the same mHags HA-1 were obtained from peripheral blood lymphocytes of three out of five individuals, each transplanted across a barrier of multiple and probably distinct mH [23]. In this regard, the most challenging observations are those of the *in vivo* expansion of T-cell subsets with selective TCR usage during acute GVHD after HLA-identical bone marrow transplantation [24•]. An anti-host T-cell clone with defined TCR Vβ usage was shown to persist in a chronic GVHD patient up until a year after HLA-identical bone marrow transplantation [25•]. These observations reveal that the mHag-specific TCR repertoire seems to be limited. Therefore the response must be directed toward only a few or a single mHag.

The production of cytokines is crucial for the induction of mHag effector cells. Without doubt, cytokines do play a significant role in the development of GVHD (for reviews see [26–28]). The inflammatory cytokines IL-1β, IL-6 and tumor necrosis factor (TNF)-α seemed to play an important role in the development of GVHD [29,30]. In a murine model, IL-1α has been postulated as a critical effector molecule in mHag-directed GVHD [31]. To my knowledge, no information exists about the contribution of cytokines to the activation of human mHag-specific effector cells in organ or bone marrow grafting.

Naturally, the impact of mHags on the outcome of organ and bone marrow grafting is dependent on, amongst other factors, their tissue distribution. Table 3 summarizes the tissues and cells we have studied to date. We have observed either ubiquitous or restricted tissue distribution of the mHags analysed (see Table 3 for details). Expression of the non-sex-linked mHags (i.e. HA-1, HA-2 and HA-5) [32], is restricted to the haematopoietic-cell lineage, including epidermal-derived Langerhans cells [33], whereas H-Y, HA-3 and HA-4 were found to be expressed on cells of all tissues tested, including cord endothelial cells and kidney proximal tubular epithelial cells [32]. Additional experiments from our laboratory showed functional expression of H-Y and HA-3 on corneal scleral tissue as well [34]. Broad tissue distribution has also been demonstrated by other investigators. Recognition of mHag on phytohemagglutinin (PHA) blasts, lymphoblastoid cell lines (LCLs) and keratinocytes was described by Niederwieser *et al.* [35] and the expression of an HLA-B35-restricted mHag on cultured kidney cells was demonstrated by Beck *et al.* [36].

Table 3

Tissue distribution of human minor histocompatibility antigens H-Y and HA-1 to HA-5.

Cell type	HA-1	HA-2	HA-3	H-Y	HA-4	HA-5
Haematopoietic progenitor cells	+	+	+	+	+	+
Clonogenic leukemia precursor cells*	+	+	+	+	+	+
Thymocytes	+	+	NT	+	NT	NT
PBLs	+	+	+	+	+	+
PBL blasts	+	+	+	+	+	+
EBV BLCL	+	+	+	+	+	+
Monocytes	+	+	+	+	+	+
Dendritic cells	+	+	+	+	+	+
Langerhans cells (skin)	+	+	+	+	+	NT
Leukaemic cells†						
myeloid	+	+	+	+	+	+
lymphocytic	+	+	+	+	+	+
Fibroblasts			+	+	+	
Keratinocytes			+	+	+	
Melanocytes			+	+		
Melanomas			+	+	+	
Cord endothelial cells			+	+		
Kidney proximal tubular epithelial cells			+	+		

*[47]. †[48]. Remaining data derived from [29] and [30]. PBLs, peripheral blood lymphocytes; EBV BLCL, Epstein–Barr virus B lymphoblastoid cell line; NT, not tested.

Of special interest are tissue-specific antigens described by Yard *et al.* [37] and Poindexter *et al.* [38•]. Both groups reported the isolation of graft-infiltrating T lymphocytes during cellular rejection, and have shown MHC class I-restricted lysis of kidney cells, but not of PHA blasts or LCLs. Moreover, kidney-specific polymorphism may exist [39]. Recently, an HLA-A3-restricted, kidney-specific, T-cell epitope was successfully isolated and sequenced; however, the origin of the protein is, as yet, uncharacterized [40••].

Clinical relevance

Most of the clinically related studies of mHags have concerned bone marrow grafting. As mentioned above, although derived from MHC mismatched donor/recipient combinations, the data on kidney tissue specific CTL clones are valuable in the understanding of the pathology of renal allograft rejection.

Several reports have described the presence of CTLs specific for anti-host mHag in patients suffering from GVHD after HLA genotypically identical BMT (reviewed in [41]). We investigated the influence of mHag H-Y and HA-1–HA-5 mismatches on the development of GVHD after bone marrow transplantation from HLA-identical donors. In a retrospective study of 148 HLA genotypically identical bone marrow donor–recipient pairs, we observed a significant correlation between mHag HA-1 mismatch and the development of GVHD in adult patients. Thus, prospective HA-1 typing would improve donor selection and identify recipients who are at high risk for GVHD [14••].

Over the past few years, evidence has accumulated to suggest that, in addition to CTLs, mHag-specific Th cells could be relevant to the pathogenesis of GVHD. *In vitro* studies have reported on host-directed Th cells in patients with GVHD (as reviewed in [41]). Th-cell responses to host mHag measured either the 'bulk' level [42] or at the Th-cell precursor level [43] correlate with GVHD. Analysis of putative mHag-specific Th-cell precursor frequencies before HLA-identical bone marrow transplantation proved to be valuable [44,45]. In a subsequent study, it was shown that both $CD4^+$ and $CD8^+$ T-cell subsets participated in the pre-transplant anti-host responses [46].

Another area of possible involvement of mHag is the so called graft-versus-leukemia (GVL) effect that, according to clinical data, parallels acute and chronic GVHD. The supposition that donor-derived mHag-specific T cells may be involved in the eradication of residual leukemic cells is attractive. One line of investigation supporting this notion is that mHag-specific CTLs are capable of inhibiting *in*

vitro outgrowth of clonogenic leukemic precursor cells [35,47], as well as lysing freshly obtained myeloid and lymphoid leukemic cells [35,48].

Besides donor-derived T cells reactive for ligands (like mHags) that are shared by host PBLs and leukemic cells, anti-host CTL responses with anti-leukemic activity *in vitro* can also be observed on either PHA blasts or leukemic cells [49–51]. This is to say that some experts argue that GVH and GVL are caused by different T-cell populations, whereas non-separable effector cells that exhibit both activities, also exist [49]. Additional detailed clinical and laboratory studies are really needed in this exciting and complicated area of clinically-related research. We also need to explain the exceptions to the rule: 'no GVHD/no relapse' and 'GVHD/relapse'.

Isolation and characterization of human mHags

In 1948, Snell [1] reported "the existence of rare h genes coded for by separate histocompatibility loci. Is there any way that these loci can be discovered? There is no simple method of finding them but that by the use of somewhat laborious methods they can eventually be brought to light".

Snell was right, mH genes can be identified, although the methodology is cumbersome. Two main lines of investigation have led to the definition of the limited number of mH peptides that are known to date. The first strategy involves the enormous task of sequencing stretches of genomic DNA known to include the genes encoding the mH T-cell epitope. Subsequent cellular testing of synthetic peptides generated according to the deduced protein sequences has resulted in the definition of peptides recognized by mH antigen-specific T-cell clones [52]. Using this approach, CTL clones defining Mta, a maternally transmitted mitochondrial murine mH antigen, were found to recognize synthetic peptides corresponding to a polymorhpic part of mitochondrial ND-1 [53]. Hydrophobic peptides of 17–26 amino acids in length were efficiently recognized, peptides of 12 amino acids were moderately well recognized, whereas shorter stretches did not sensitize target cells for recognition by anti-Mta CTLs at all.

Demotz *et al.* [54] were the first to isolate and characterize naturally processed peptides from MHC molecules. Rammensee and his colleagues successfully applied immunopurification and biochemical isolation techniques to extract murine mH peptides from class I molecules. As was true for other T-cell recognized antigens, such as viral [55] and non-viral proteins [54], they found that the murine mHags H-Y and H-4 were naturally processed proteins, probably of a peptidic nature [56,57].

Sekimata *et al.* [58] and De Bueger *et al.* [59] managed to isolate fractions containing mHag peptides, but failed to obtain the actual amino-acid sequence from the peptide pool eluted from MHC class I molecules. Thanks to the technical advances of Hunt *et al.* [60], the application of a microcapillary HPLC-electrospray ionization tandem mass spectrometry enabled the detection of non-abundant peptides among a pool of MHC-bound peptides. Our joint forces allowed the first identification of two classical mHag: the human mHag HA-2 and the male-specific mHag H-Y [16••,61••]. The HA-2 peptide most probably originates from a member of the non-filament-forming class I myosin family, a large family of proteins that are involved in cell locomotion and organelle transport [62]. The H-Y antigen presented by HLA-B7 is an 11-residue peptide derived from SMCY, an evolutionarily conserved protein encoded by the Y chromosome [63••]. Besides the role of H-Y as a transplantation antigen, the human Y gene controlling the expression of the HLA-B7-restricted mHag H-Y T-cell epitope is possibly also functioning as a gene controlling spermatogenesis [64]. Concurrent with the identification of the human H-Y peptide, a murine H-Y peptide was characterized and appeared to be derived from the same evolutionarily conserved SMCY protein [63••].

Besides the identification of other class I-restricted human mHag peptides, we are currently aiming at characterizing the biochemical nature of MHC class II-restricted human mHags. In view of the limited information on their genetics and polymorphisms, it is of particular interest to find out whether the class II mHags belong to the small number of cytosolic proteins that have deviated to the endosomal processing pathway.

Conclusions

Significant information has been gathered over the past few years on human mHags. Although the number of mH systems is expected to be large, probably only a limited number will fulfil the necessary criteria (i.e. frequency, immunogenicity and tissue distribution) for being a risk factor for GVHD or rejection. Dissection of the major from the minor minors and their biochemical identification may aid in immunomodulatory approaches. The potential applications vary from tolerance induction in organ and bone marrow grafting, to the design of prophylaxis against GVHD and rejection. Most promising is immunotherapy using CTLs specific for mHag peptide for the treatment of residual, refractory or relapsed leukemia. Finally and less far-fetched, as more mHags become biochemically identified, their use for diagnostic in bone marrow donor selection by molecular typing.

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