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MINOR HISTOCOMPATIBILITY ANTIGEN MATCHING: ACTUAL FACT OR WISHFUL THINKING?

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Graft-versus-Host-Disease (GvHD) still forms a major barrier to successful Bone Marrow Transplantation (BMT) between HLA matched as well as between HLA identical BM donor/recipient pairs. It is well believed that disparities for minor Histocompatibility antigens (mHag) between BM donor and recipient create a potential risk for GvHD (1,2).

Assuming that as in the mouse, the human genome has an abundancy of mH loci, identification of the "majors" under the "minors" is a prerequisite. A 'major' minor distinghuishes itself through its population frequency, immunodominancy and tissue distribution. These prerequisites have been analysed lately by our group for a small number of mHag. Here we will briefly summarize the results of these studies and discuss them in view of the clinical data. In addition, new aspects on the nature of human mHag will be discussed as well.

Immunogenetic studies of five non-sexlinked mHag (designated HA-1 to HA-5) revealed that some mHag appeared frequent (69-95%), others occurred with lesser frequencies (7-16%) in the healthy population (3). It should be noted that mHag HA-1 has a phenotype frequency of 69% in the population, so an HA-1 mismatch between HLA identical or matched donor/recipient combinations is likely to occur. An analysis of the genetic traits of mHag HA-1 to HA-5 demonstrated that they segregate in a Mendelian fashion. Each of them can be considered as a product of a single gene with two alleles, one expressing and one not expressing the detected specificity. The loci encoding the mHag are not closely linked to HLA (4).

Three sets of data are indicative for a hierarchy in *immunogenicity* among mHag. *Firstly*, CTL clones reactive to the same mHag HA-1 were obtained from peripheral blood lymphocytes of 3 out of 5 individuals each transplanted across a multiple and probably distinct mH barrier (see table 1 and ref. 3).

Table 1

MINOR H ANTIGEN SPECIFIC CTL CLONING

Patient CTL line	N. of clones	mHag	% pop. freq.
patient 1	**N=7**	**HA-1**	**69%**
HA-1	N=9	HA-?	
patient 2	N=17	HA-2	95%
HA-2	N=20	HA-?	
patient 3	N=27	HA-3	88%
HA-3	N=20	HA-?	
patient 4	N= 8	HA-4	16%
HA-4	**N=10**	**HA-1**	**69%**
	N=11	HA-?	
patient 5	N= 4	HA-5	7%
HA-5	**N=11**	**HA-1**	**69%**
	N=16	HA-?	

Secondly, we analysed the composition of the T cell receptor (TCR) V regions of twelve of the latter HLA-A2 restricted mHag HA-1 specific CTL clones derived from three unrelated patients suffering from GvHD. Most surprising, the Vß regions, but not the TCR Vα and Jα genes, of the TCR specific for the HLA-A2/mHag HA-1 complex expressed by all twelve CTL clones share extensive sequence homologies (5). These observations support our notion on the (in vitro) immunogenic potency of the MHC/mHag HA-1 complex. Moreover, we noticed a limited TCR repertoire usage among the CTL clones analysed. Several CTL clones, obtained from independent limiting dilutions and from different unrelated patients, shared fully identical TCR α and ß sequences (5), which could be indicative for a dominant mHag specific T cell response occurring during the development of GvHD post-BMT.

Thirdly, in an international collaborative[1] study, comprising 148 bone marrow HLA identical donor/recipient pairs, investigating the influence of mHag HA-1 to HA-5 mismatching on the development of GvHD, we observed a significant correlation between mHag HA-1, -2, -4 and -5 mismatch and GvHD (6). In view of the population

frequency of HA-1, analysis of solely mHag HA-1 mismatch and occurrence of GvHD in adult patients appeared significant (table 2).

Table 2.

Correlation of mHag HA-1 with the occurrence of GvHD

Adult patient		mHag	
		match	mismatch
GvHD	No	22	0
	Yes	49	10

P = 0.033

Clinical relevance of anti-host CTLs in the pathogenesis of GvHD. Bone marrow derived T lymphocytes are responsible for anti-host mHag specific reactivities. Little is known about the basis of the T cell responses mHag evoke in BMT, although controversies regarding the role of anti-host CTLs in the development of GvHD after BMT do exist. In our earlier studies, we demonstrated the presence of anti-host mHag specific CTLs in the blood of patients undergoing GvHD. Although patients with chronic GvHD tended to develop higher and more persistent levels of anti-host CTL activity than those without GvHD, this finding was not statistically significant (7). Subsequent analyses at the quantitative level i.e. determination of the frequencies of anti-host specific CTL precursors revealed high frequencies of mHag specific CTLs detectable in the blood early after BMT irrespective of the GvHD status of the patient (8). We have therefore, recently started to investigate, whether we could find qualitative differences in the anti-host CTLs in patients with and without GvHD. Based on earlier studies in kidney grafting where the in vitro resistance in a Mixed Lymphocyte culture response for the immunosuppressive drug Cyclosphorine A (CsA) was correlated with a higher rate of graft loss (9), we analysed the in vitro sensitivity of the anti-host CTLs for CsA. Patients (N = 12) were divided into different groups according to their GvHD status. PBLs were isolated before and at several dates after HLA genotypically identical BMT and used to determine CTLp frequencies of each patient in limiting dilution assays, with and without the addition of CsA to the test medium. The CTLp frequency of patients without GvHD could all be inhibited very well by CsA at all dates. However, the CTLp frequency in patients with acute GvHD could not be inhibited by CsA at most dates after transplantation. The CTLp frequency of patients with chronic GvHD could in some cases not be inhibited, in others it was inhibited, but always less than in no GvHD patients. Sofar, our results show that CsA resistant CTLs are mainly found in patients suffering from (acute) GvHD. This strongly suggests that they play an important role in the pathogenesis of GvHD (10). Our future aim is to confirm this phenomenon by analyzing more patients and to characterize these CsA resistant CTLs.

Towards the nature of human mHag. Naturally, an answer to the question what mH antigens are is really needed. It would not only reveal their physiological nature but more importantly provide inside into their putative role in organ and bone marrow transplantation. We therefore aimed at the biochemical characterisation of human mH antigens. Hereto, we made use of the immunopurification and biochemical techniques succesfully applied by Rammensee and his colleagues to extract murine mH peptides from MHC molecules. Indeed, HPLC separation of low Mr molecules (< 10 kD) obtained from acid treated MHC class I HLA-A2.1 molecules appeared successful. Fractions with sensitizing activity for the non-sexlinked mH antigen HA-2 specific CTL clones were isolated (11). Our observations are in line with previous reports on the isolation of naturally occurring peptides that represented classical murine mH antigens i.e. H-Y and H-4 (12, 13). Similar to our own results, Sekimata et al. isolated an human mH antigenic peptide from EBV-LCLs by acid elution. This HLA-B35 restricted mH antigen was earlier shown to play a role in HLA identical kidney graft rejection (14). Further characterization i.e. exact amino acid sequence and identity of the protein from which murine or human mH antigens originate remain to be determined.

Although the amino acid is not available yet, we do

know that mHag HA-1 and HA-2 are of *peptidic nature*. Two sets of data underline this statement. First, the sensitizing activity of the mHag containing fractions, obtained as described above, are susceptible to protease treatment (15); i.e. incubation of these mHag containing HPLC fractions with pronase or proteinase K abolished the sensitizing activity. Second, the MHC encoded TAP1 and TAP2 gene products are required for mHag peptide presentation on the cell surface. The transporter genes TAP1 and TAP2 associated with antigen presentation are required for delivery of peptides from the cytosol with the endoplasmic reticulum (16). The availibility of a human celline "T2" lacking both transport and proteasome subunit genes enabled us to study the processing and presentation of human mH antigens. We demonstrated that the (rat) transport gene products TAP1 and TAP2^a were required for processing and presentation of antigenic peptides from influenza virus and from the intracellular mH protein HA-2 (table 3 and ref. 17). It must be noted that reconstitution and thus presentation of mHag HA-2 to its relevant T cell clone was performed with the rat TAP2^a allele. As can be seen from table 3 restoration of presentation of mHag HA-1 did not occur. In the rat, polymorphism of the TAP genes have been observed (18). Subsequent cotransfection into the T2 cell line of the rat TAP2^u (cim b) allele instead of the TAP^a (cim a) allele resulted in presentation and thus recognition of mHag HA-1 (and HA-2) on the cell surface (table 3).

Table 3

mHag require TAP gene products for their presentation

Target cells	Effector cells			
	A2.1	HA-1	HA-2	Q 66.9*
T1	96°	68	71	62
T2	100	3	0	8
T2/TAP1	100	1	0	12
T2/TAP2	81	1	5	5
T2/TAP1+2^a	82	7	57	33
T2/TAP1+2^u	78	71	73	n.t.

* influenza virus specific
° % lysis at an effector/target ratio of 10 : 1

Taken together, these results show that mHag HA-1 and HA-2 are cytosolic peptides and require TAP gene products to be transported over the endoplasmic reticulum where they associate with newly sensitized class I molecules. It also demonstrate that HA-1 and HA-2 are different peptides. Powis et al. (18) showed the effect of TAP polymorphism on the peptide composition. Reverse-phase HPLC analysis of peptides eluted from TAP^a versus TAP2^u molecules demonstrated different peptide profiles.
In conclusion, although the number of mH systems is expected to be large, probably only a limited number will fulfil the criteria (i.e. frequency, immunogenicity, tissue distribution) for being a risk factor for GvHD or rejection. To dissect the majors from the minor minors yet more information is needed on the Th and CTL defined human mH antigens repertoire in order to establish the immunodominant ones. To understand their biological role in bone marrow transplantation information on their cytokine secretation profiel is also essential. Finally, once the nature of the human mHag is established the search for immunological relevant mH families will hopefully accelerate.

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