"The role of human minor Histocompatibility antigens in graft failure; a mini-review."

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THE ROLE OF HUMAN MINOR HISTOCOMPATIBILITY ANTIGENS IN GRAFT FAILURE: A MINI-REVIEW

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This brief review will summarise our present knowledge on the possible role of human minor histocompatibility antigens (mHags) in graft failure. Following a short introduction on the possible nature of human mHags some *in vitro* studies dealing with graft failure and rejection will be discussed. To understand the possible impact of mHag disparity between organ donor and recipient on the outcome of organ and bone marrow (BM) grafting, information on their tissue expression is essential; hence a summary is given on the mHag tissue distribution studies performed so far. Finally, we will present our preliminary studies on the expression of mHag on human corneal tissue.

POSSIBLE NATURE OF HUMAN mHag

mHags are most probably naturally processed peptides of cytosolic proteins.[1,2] The MHC restricted presentation of mHag peptides on the cell surface requires peptide import by an ABC transporter dependent system into the endoplasmic reticulum, where they bind to newly sensitised MHC molecules.[3] In the clinical setting of organ and BM transplantation between HLA matched, mHags mismatched individuals, the mHags are capable of inducing vigorous immune responses leading to graft rejection or graft-versus-host disease.[4–6] The male-specific H-Y is by far the simplest and also the most extensively studied mHag The first report on H-Y as a transplantation antigen is an untitled communication by Eichwald and Silmser in 1955. These authors observed that within two inbred strains of mice, most of the male-to-female skin grafts were rejected, whereas transplants made in other sex combinations nearly always succeeded.[7] The term H-Y antigen was introduced by Billingham and Silvers because the male-specific antigen can function as a classical transplantation antigen responsible for homograft rejection.[8]

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mHag AND GRAFT FAILURE: SOME RELATED CLINICAL STUDIES

In the human situation the first report on involvement of H-Y in transplantation appeared in 1976.[9] It concerned a clinical observation of rejection of a BM graft from a male sibling by his HLA-identical sister. *In vitro* analysis of the post-transplant peripheral blood lymphocytes (PBLs) of this female patient (HLA phenotype: HLA-A2, -A2, -B44, -B60, -Cw3, -Cw5, -DR4, -Drw6) showed unambiguously strong cytotoxic T cell (CTL) responses specific for *male* HLA-A2 positive target cells.[9,10] Whether the H-Y specific CTLs actually mediated the allograft rejection, we do not know. It must be remarked, however, that most probably the female patient, who suffered from severe aplastic anaemia, had been sensitised to the H-Y antigen prior to BM transplantation through multiple, mainly male, blood transfusions and pregnancies. This assumption is based on our subsequent observations. As shown in Table I, PBLs derived from four additional cases showed, after *in vitro* restimulation with HLA-identical male cells, exactly the same phenomenon, namely HLA restricted (-A1, -A2 and/or -B7) anti-H-Y CTL activity. In one patient (i.e. case 5, Table I), the H-Y specific HLA-B7 restricted cytotoxicity was detected shortly after an acutely rejected kidney donated by an HLA-identical male sibling (unpublished observation). In circumstances similar to ours, other investigators also described the presence of HLA restricted H-Y directed cytotoxicity.[11,12]

Although in our first case we could not formally prove that the H-Y specific CTLs actually mediated the rejection of the male BM allograft, some years

Table I. MHC restricted cytotoxic T cell (CTL) responses against the mHag H-Y

Patient/disease[a]	CTLs	CTL specificity
1. AA	Post BM grafting	HLA-A2 H-Y
2. AA	Multitransfused	HLA-A2 H-Y
3. AA	Multitransfused	HLA-A2 H-Y/HLA-B7 H-Y
4. AA	Multitransfused	HLA-A1 H-Y
5. Kidney failure	Post renal transplant	HLA-B7 H-Y

AA, aplastic anaemia; BM, bone marrow.
[a] All patients were female.

ago we were confronted with a case with a fatal outcome in which anti-H-Y CTLs were most probably mainly responsible for BM graft failure. It concerned a multitransfused female patient suffering from myelodysplasia after treatment for Hodgkin's disease. *In vitro* analysis prior to BM transplantation demonstrated the presence of HLA-A1 restricted anti-H-Y CTLs (Table I, case 4). Since the father appeared to be the only HLA compatible related donor, he was the obvious choice (despite the presence of the patient's pretransplant anti-H-Y CTLs). Notwithstanding intensive pretransplant immunosuppressive treatment and the donation of T cell depleted marrow, there was no recovery of the bone marrow haematopoietic function.[13] In view of the latter case, expression of mHag on haematopoietic stem cells (HPC) might be relevant in presensitised patients receiving a mHag-positive T cell depleted marrow graft. For that purpose, the expression of the male specific antigen H-Y was studied for its expression on HPC. It became clear that indeed H-Y is expressed on CFU-GEMM, CFU-GM and BFU-E.[14] Experiments carried out to study the expression of other (non-sex-linked) mHag (designated HA-1 to HA-5), demonstrated expression of all the latter antigens on HPC.[14,15]

The clinical relevance of the H-Y alloantigen in the context of HLA-A2 to the results of human kidney allograft transplantation has also been determined. A retrospective study showed that HLA-A2 females receiving HLA-A2 male kidneys survived for a significantly shorter time than did non-HLA-A2 male kidneys in non-HLA-A2 female recipients.[16]

mHag TISSUE DISTRIBUTION STUDIES

Naturally the impact of mHags on the outcome of organ and BM grafting is dependent on, amongst other things, their tissue distribution. Table II summarises the tissues and cells studied to date. We observed ubiquitous versus restricted tissue distribution of the mHags analysed.[17] Expression of the non-sex-linked mHags, i.e. HA-1, -2 and -5,[17] is restricted to the haematopoietic cell lineage including epidermal-derived Langerhans cells,[18] whereas H-Y, HA-3 and HA-4 were found to be expressed on cells of all tissues tested (see Table II).

The contribution of mHags to the cascade of inflammatory events, especially in cutaneous allograft rejection, is largely dependent on which type of antigen presenting cell (APC) the antigen is presented on. Namely, T cells can be inactivated instead of activated when occupation of their T cell receptor (TCR) by antigen is not accompanied by appropriate co-stimulatory signal(s).[19] This so-called state of T cell anergy is marked by unresponsiveness to subsequent adequate triggering by professional APC and can be induced in several ways.[19] Keratinocytes, fibroblasts, and many other

Table II. Tissue distribution of human minor histocompatibility antigens

	HA-1	HA-2	HA-3	H-Y	HA-4	HA-5
Haematopoietic stem cells	HA-1	HA-2	HA-3	H-Y	HA-4	HA-5
Thymocytes	HA-1	HA-2	n.t.	H-Y	n.t.	n.t.
Peripheral blood lymphoctyes	HA-1	HA-2	HA-3	H-Y	HA-4	HA-5
PBL blasts	HA-1	HA-2	HA-3	H-Y	HA-4	HA-5
EBV BLCL	HA-1	HA-2	HA-3	H-Y	HA-4	HA-5
Monocytes	HA-1	HA-2	HA-3	H-Y	HA-4	HA-5
Dendritic cells	HA-1	HA-2	HA-3	H-Y	HA-4	HA-5
Leukaemic cells						
Myeloid	HA-1	HA-2	HA-3	H-Y	HA-4	HA-5
Lymphocytic	HA-1	HA-2	HA-3	H-Y	HA-4	HA-5
Langerhans cells (skin)	HA-1	HA-2	HA-3	H-Y	HA-4	n.t.
Fibroblasts			HA-3	H-Y	n.t.	
Keratinocytes			HA-3	H-Y	HA-4	
Melanocytes			HA-3	H-Y		
Melanomas			HA-3	H-Y	HA-4	
Cord endothelial cells			HA-3	H-Y		
Kidney proximal tubular epithelium cells			HA-3	H-Y		

PBL, peripheral blood lymphocytes; EBV BLCL, Epstein–Barr virus B lymphoblastoid cell line; n.t., not tested.

Table III. mHag expression on human corneal tissue: preliminary results

Cornea donor[a] HLA type	mHag specific clones			
	*A1*HY[b]	*A1*HA-3	*A2*H-Y	*B7*H-Y
Experiment 1				
HLA-A3 (F)	0	n.t.	n.t.	7[c]
HLA-A1 (M)	0	36	n.t.	n.t.
HLA-B7 (M)	n.t.	n.t.	n.t.	16
Experiment 2				
HLA-A1 (M)	14	47	n.t.	n.t.
HLA-A1 (F)	0	32	n.t.	n.t.
	HLA specific and mHag specific clones			
	HLA-*A1*[d]	*A1*HA-3	HLA-*A2*	*A2*H-Y
Experiment 3				
HLA-A1 (M)	59	45	n.t.	n.t.
HLA-A2 (M)	n.t.	n.t.	45	71

(M), male; (F), female; n.t., not tested.
[a]Corneal scleral tissue incubated with 200 U/ml IFNγ for 48 hours, trypsinised, resuspended and used as target cells in the cell mediated lympholysis (CML) assay.[17]
[b]mHag specific CTL clones are used as effector cells. The mHag HY can be recognised in the context of different HLA molecules (see Table 1 and ref. 4). The recognition of mHag HA-3 is restricted to HLA-A1.[4]
[c]Percentage specific lysis in the CML assay: <10% is negative, >11% is scored as positive.
[d]HLA specific CTL clones used in this study are directed against the HLA molecules HLA-A1 and HLA-A2.

so-called non-classical APC[20] have been found to have no or little capacity of T cell activation, even after interferon-gamma induced HLA class II expression.[21,22] HLA class II expressing keratinocytes (also found in a GVHD-affected skin,[20] were even found to tolerise hapten-specific T cell clones *in vitro*.[23]

The expression of mHag on non-classical APCs, such as HA-3 on keratinocytes, could play a role in the induction of BM transplantation tolerance. We earlier investigated the development of acquired tolerance for mHag HA-3 in a healthy chimaeric BM recipient 7 years after HLA-identical but HA-3 mismatched BMT.[24] We found persistent host specific HA-3 expression on patient's skin tissue after BM transplantation together with disappearance of anti-host HA-3 CTLs which paralleled the *in vivo* state of tolerance. We hypothesised, therefore, that direct presentation of host mHags by parenchymal host tissues functioning as non-classical or inadequate APC could result in tolerisation of anti-host CTLs induced following BM engraftment.[24] According to this hypothesis, the induction of long-term graft–host tolerance versus graft–host reactivity after BM transplantation across mHags barriers would depend on the tissue distribution of the mHags in question.

Similarly, the need for adequate signals for T cell activation and subsequent murine corneal allograft rejection was earlier put forward by Chandler *et al.*[25] Indeed, inadequate presentation of the mHag H-Y in a murine cornea model led to a specific state of unresponsiveness to H-Y.[26] On the other hand when the corneal graft, which is normally devoid of Langerhans cells (LC), became infiltrated with donor-derived LC through graft pretreatment with latex beads, rejection of mHag-disparate corneal grafts was observed.[27] Although, as discussed above, keratinocytes are not capable of inducing primary T cell activation, they do have limited capacity to activate memory T cells.[28] In this context it is of importance to note that allogeneic corneas that were transplanted into eyes of presensitised mice were uniformly subjected to an acute rejection process.[29] With regard to the role of mHag in the latter study, it was shown that the highest rate of rejection occurred among grafts that confronted their hosts with multiple mHag, with or without major histocompatibility antigens.[29]

mHag EXPRESSION ON HUMAN CORNEAL TISSUE: PRELIMINARY RESULTS

It is well known that corneal tissue recipients do benefit from an HLA matched graft,[30] especially the high-risk patients.[31] Even in those HLA matched cases, corneal graft survival, after excluding the non-immunological causes for graft failure, is well below 100%.[32] Since HLA antigens are expressed on corneal tissue,[33] it would, in view of their characteristics, not be surprising that mHags will coexist as well. To elaborate on the latter assumption, we recently started to analyse the functional expression of human mHags on corneal scleral tissue. Our well-defined CTL clones specific major HLA antigens HLA-A1 and HLA-A2 and for the mHag H-Y (see Table I) and for the non-sex-linked mHag HA-3[4] were used as effector cells in cell mediated lympholysis (CML) assays. As target cells, trypsinised corneal scleral tissues were used. Table III demonstates the results of our first small series of experiments. Both HLA antigens and mHag H-Y and HA-3 are readily detectable on corneal tissue; they function as target molecules for CTL clones. These observations are in agreement with our earlier

studies on the broad mHag tissue expression of H-Y and HA-3. Likewise, it is to be expected (Table II), but has yet to be proven, that *all* mHag we can test for to date are expressed on LCs residing in the corneal epithelium. This knowledge is especially important in view of the LC's characteristic as an adequate APC.

We demonstrate here for the first time, to our knowledge, the functional expression of mHags on human corneal tissue. Naturally these limited data need confirmation and extension. The present studies may bring us closer to understanding the impact of mHag mismatching between corneal graft donor and recipient in the human situation. The role of mHag disparities, in especially presensitised recipients, was recently clearly demonstrated in a murine study.[29] Encouraged by the latter data we feel it is justified to continue our search for mHags on corneal tissue and their possible role in the outcome of corneal grafting.

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