Lifting a tip of the veil of human minor Histocompatibility antigens.

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Human bone marrow transplants performed as therapeutical treatment of severe aplastic anaemia, leukaemia and immune deficiency disease became available in the seventies (Bortin, 1970). In an artificial situation, such as organ transplantation, the major Histocompatibility (H) antigens function as a major transplantation barrier and thus play an important role in the survival of transplants and patients. Consequently, improved success in bone marrow transplantation was reported when matching for the HLA antigens was taken into account (Thomas, 1975). Between 1975 and the present day, the long-term results of allogeneic bone marrow transplantation (BMT) have greatly improved due to the use of HLA-matched siblings as marrow donors, advanced pretransplant chemoradiotherapy, the use of potent immunsuppressive drugs as Graft-versus-Host-Disease (GvHD) prophylaxis, better antibiotics and isolation procedures.

The results of clinical bone marrow (BM) transplantation reveal however that the selection of MHC identical donors/recipients is not a guarantee of avoidance of GvHD or disease free survival even when donor and recipient are closely related (Bortin, 1991). It is believed that disparities for minor Histocompatibility antigens (mHag) between donor and recipient constitute a potential risk for GvHD or graft failure (Martin, 1991; Beatty, 1989).

In man, the efforts of several investigators have led to the identification of a small number of mHag (Goulmy, 1977, 1983; Zier, 1983; Irlé, 1990; Van Els, 1992). Here, we will briefly focuss on their possible clinical relevance for BM transplantation in both the GvHD and the Graft-versus-Leukemia (GvL) reactivities. Furthermore, we provide preliminary evidence for the evolutionary conservation of human mHag.

Clinical relevance of anti-host cytotoxic T cells (CTLs) and helper T cells (Th) in the development of GvHD

Several reports demonstrated the presence of anti-host mHag specific CTL in patients suffering from GvHD after HLA genotypically identical BMT (Goulmy, 1983; Tsoi, 1980, 1983; Irlé, 1985; Van Els, 1990; Irscheck, 1992; Niederwieser, 1993). In our laboratory,

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much effort was put into the further characterization of a (small) number of anti-host mHag specific CTLs. Hereto, CTL clones specific for host mHag were isolated from the peripheral blood (PBL) of patients suffering from severe GvHD. Subsequent immunogenetic analyses revealed that these CTL clones identified five non-sexlinked mHag, designated HA-1, -2, -3, -4, -5, which are recognized in a classical MHC restricted fashion (Van Els, 1992). mHag HA-3 is recognized in the presence of HLA-A1 and mHag HA-1, -2, -4 and 5 require the presence of HLA-A2. In order to document the effect of mH antigens in genotypically identical BMT on the occurrence of acute (grade ≥ 2) GvHD, we prospectively collected PBL from HLA-A1 and HLA-A2 positive patient/donor sibling pairs. This multi center study comprised 148 HLA genotypically identical BM donor/recipient combinations, adults as well as children, grafted between 1982 and 1990. The results of the mHag typing using the CTL clones specific for five well defined mHag HA-1 to HA-5 demonstrated (table 1) a significant correlation between mHag HA-1, -2, -4 and -5 mismatch and GvHD (Goulmy, 1994).

Table 1 Correlation of mHag HA-1, -2, -4 and -5, with the occurrence of GvHD

All patients		mHag	
		match	mismatch
GvHD	No	41	1
	Yes	40	12

P = 0.002

The last few years evidence has accumulated that in addition to CTLs mH antigen specific helper T cells (Th) could be relevant in the pathogenesis of GvHD. In vitro studies reporting on host directed Th cells have been described in patients having GvHD (Tsoi, 1980; Reinsmoen, 1984; Irlé, 1987). Van Els et al. reported on the long term kinetics of Th cells in response to host mH antigens in 16 patients and demonstrated that significant Th cell activity in vitro correlates with clinical acute GvHD (Van Els, 1990b). In a subsequent study, we demonstrated that these anti-host Th cells carry the CD4 phenotype and recognize mH antigens in the context of HLA-DR and -DP (Van Els, 1990c).

Most recent observations support the notion that mH antigen specific Th cells are by and large likely to play a role in the pathogenesis of acute GvHD (Theobald, 1992; Schwarer, 1993). In both latte activities have been measurec

Possible involement of mHag

The hypothesis that posttrans have a beneficial effect is bas potential as a 'desired' side-e Weiden, 1981a, 1981b). In sea observed earlier both absence clinical signs of GvHD (see ta

Table 2 Anti-host T cell activ

	An C +++
no GvHD	5
acute GvHD	6
chronic GvHD	15

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It was notable that in 3 out leukemia relapse was manifes in the absence of GvHD (N-5 leukemic activity. In an atter their putative anti-leukemic a related acitivities. The latter t only. The former type of CT shared by host PBL and investigation support the not the anti-leukemic effect of all of inhibiting in vitro outgrowt freshly obtained myeloid an Harst, 1994).

Schwarer, 1993). In both latter studies the primary in vitro putative mH antigen Th activities have been measured by IL-2 production of the responding cell population.

Possible involement of mHag in Graft versus Leukemia

The hypothesis that posttransplantation of bone marrow anti-host CTL activity may have a beneficial effect is based on the assumption of the postulated anti-leukaemic potential as a 'desired' side-effect of the post BMT complication GvH. (Bortin, 1973; Weiden, 1981a, 1981b). In search for anti-host CTL and Th cell activities post BMT, we observed earlier both absence and presence of anti-host CTL in patients without any clinical signs of GvHD (see table 2).

Table 2 Anti-host T cell activities after HLA identical BMT.

	Anti-host CTLs		Anti-host Th	
	+++	---	+++	---
no GvHD	5	4	2	5
acute GvHD	6	3	6	0
chronic GvHD	15	1	5	2
	N = 34		N = 20	

It was notable that in 3 out of 4 cases without anti-host CTL activity (see table 2), leukemia relapse was manifested. On the other hand, the presence of anti-host CTLs in the absence of GvHD (N-5) argues for the possible role of these CTLs in the anti-leukemic activity. In an attempt to study the post BMT anti-host CTL responses for their putative anti-leukemic activity in vitro, we observed "GvHD" related and "GvL" related acitivities. The latter type of CTL clones recognized patient's neoplastic cells only. The former type of CTL clones were reactive with ligands, like mH antigens, shared by host PBL and leukemic cells (Van Lochem, 1992). Another line of investigation support the notion that anti-host mHag specific CTL may play a role in the anti-leukemic effect of allogeneic BMT. Namely, mHag specific CTLs are capable of inhibiting in vitro outgrowth of clonogeneic leukemic precursor cells as well as lyse freshly obtained myeloid and lymphoid leukemic cells (Fakenburg, 1991; Van der Harst, 1994).

Human mHag are conserved in evolution

To substantiate the importance of the human mH antigenic systems, we investigated whether the mHag are conserved in evolution between man and chimpansee. Hereto, cells from chimpansees were transfected with the human HLA-A2.1 gene. Subsequent analyses with our human allo HLA-A2.1 and four mHag HLA-A2.1 restricted CTL clones revealed the presentation of chimpansees' allo and mHag peptides in the context of the transfected human HLA-A2.1 molecule by chimpansees' target cells (table 3). These results implicate that the chimpansee cell derived allo and mHag peptides investiged in this study are very similar to the human allo HLA-A2 and HLA-A2 restricted mHag peptides.

Table 3 Human mHag are evolutionary conserved

Chimp. target cells*		Human CTL clones				
		allo A2	A2HY	A2 HA-1	A2 HA-2	A2 HA-4
Theo	♂	76°	79	69	75	3
Japie	♂	48	58	45	54	0
Pearl	♀	37	2	35	40	2
Debbie	♀	42	1	37	48	1
Gwen	♀	23	0	38	32	1
Brigitte	♀	31	0	23	20	0
Sherry	♀	52	2	58	55	3

* chimp. target cells have been electroporated with the HLA-A2 gene.

° % specific cytotoxicity measured in a cell mediated lympholysis assay.

In conclusion, although lots of information was gathered during the past decades on the murine and human mHag, still many questions remain to be answered. Besides identification of the mHag and the genes they are encoded by, we must be able to dissect the majors from the minor minors. To achieve this, more information is needed on the Th and CTL defined human mHag repertoire, and to establish the immunodominant ones. To understand their biological role in bone marrow transplantation information on their cytokine secretion profile is essential.

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