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 immunosuppressive 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; Irle, 1990; Van Els, 1992). Here, we will briefly focus 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; Irle, 1985; Van Els, 1990; Irscheck, 1992; Niederwieser, 1993). In our laboratory,
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 Eis, 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

<table>
<thead>
<tr>
<th></th>
<th>mHag match</th>
<th>mHag mismatch</th>
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<tbody>
<tr>
<td>All patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>No GvHD</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Acute GvHD</td>
<td>15</td>
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<td>Chronic GvHD</td>
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</table>

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 (Tsui, 1980; Reinsmoen, 1984; Irlé, 1987). Van Eis 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 Eis, 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 Eis, 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 latter activities have been measured.

Possible involvement of mHags

The hypothesis that putative anti-leukemia activities have a beneficial effect is based on the observation that posttransplantation leukemia relapse was manifested in the absence of GvHD (N-5). In an attempt to investigate their putative anti-leukemic activities, the latter type of CT cell activity was measured. The former type of CT cell activity, related to the anti-leukemic effect of all of inhibiting in vitro outgrowth of freshly obtained myeloid an leukemic cells, was measured. In both latter activities have been measured.

Possible involvement of mHags

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of a (small) number of anti-host host mHag were isolated from severe GvHD. Subsequent studies identified five non-sexlinked antigens in a classical MHC recognized in the presence of HLA-A2. In order to identical BMT on the occurrence PBL from HLA-A1 and HLA-A2 study comprised 148 HLAs, adults as well as children, typed using the CTL clones a significant th and GvHD (Goulmy, 1994).

the occurrence of GvHD

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Possible involvment 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.

<table>
<thead>
<tr>
<th>Anti-host CTLs</th>
<th>Anti-host Th</th>
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<tr>
<td>++ + H</td>
<td>+++ Th</td>
</tr>
<tr>
<td>no GvHD</td>
<td>5 4 2 5</td>
</tr>
<tr>
<td>acute GvHD</td>
<td>6 3 6 0</td>
</tr>
<tr>
<td>chronic GvHD</td>
<td>15 1 5 2</td>
</tr>
</tbody>
</table>

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 GvH (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 activities. 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 investigated in this study are very similar to the human allo HLA-A2 and HLA-A2 restricted mHag peptides.

<table>
<thead>
<tr>
<th>Table 3: Human mHag are evolutionary conserved</th>
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<tr>
<td><strong>Chimp. target cells</strong></td>
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<td></td>
</tr>
<tr>
<td>Theo</td>
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<tr>
<td>Japie</td>
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<tr>
<td>Pearl</td>
</tr>
<tr>
<td>Debbie</td>
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<tr>
<td>Gwen</td>
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<tr>
<td>Brigitte</td>
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<td>Sherry</td>
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</tbody>
</table>

* 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.

**Acknowledgements**

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Goulmy E, Gratama JW, Bortin MM, et al. (1986) Transplant Proc. 23: 61-

Irlé C, Beatty PG, Mickelson DJ et al. (1986) Transplant Proc. 23: 61-


Irscheck E, Hladik T, Niederkorn JY, et al. (1986) Transplant Proc. 23: 61-
In 1991, we investigated human and chimpanzee. Hereto, we analyzed HLA-A2.1 gene. Subsequent studies revealed that HLA-A2.1 restricted CTL and mHag peptides in the chimpanzees' target cells, which were derived allo and mHag human allo HLA-A2 and HLA-A2.1 gene.

During the past decades, much to be answered. Besides led by, we must be able to gather more information is needed. Therefore, and to establish the crucial role in bone marrow allo immunocompetent cells, Transplantation 9: 571-587.

Acknowledgements
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Compatibility antigens as a transplantations from unrelated ow Transplantation. 8: 217-223.

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