RECOGNITION OF AN - AS YET UNKNOWN - MINOR TRANSPLANTATION ANTIGEN BY POST-TRANSPLANT LYMPHOCYTES FROM AN A.M.L. PATIENT

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SUMMARY

The occurrence of a severe GvHD in a bone marrow transplanted male A.M.L.-patient prompted us to investigate the in vitro activity of cytotoxic T cells of patients' lymphocytes post-transplantation. The male A.M.L.-patient had been transplanted with an HLA identical female sibling donor. Full chimerism developed. Patients' lymphocytes post-transplantation were stimulated with pre-transplant patients' lymphocytes. The 6 days effector cells were tested in a Cell Mediated Lympholysis assay against randomly selected panel- and familydonors. Strong cytolytic activity was observed against an antigen which is also present on patients' own pre-transplant lymphocytes.

Preliminary findings show that the patients' effector cells recognize an as yet unknown target determinant which is associated with HLA-A2 in a randomly chosen panel of unrelated individuals and which shows Mendelian segregation. The recognition of this determinant is HLA-dependent but the location of the locus coding for it is unknown.

INTRODUCTION

Graft-versus-host reactions (GvHR) are probably caused by subpopulations of donor T cells which differ for histocompatibility antigens (1). It is also known that positive cytotoxic tests can be found before and after bone marrow grafting between HLA identical siblings (2-4). Naturally, these observations can be attributable to non Major Histocompatibility Complex (MHC) antigens. We reported earlier on 3 cases by which the peripheral blood lymphocytes of female aplastic anaemia patients were able to show HLA-restricted H-Y killing in a Cell Mediated Lympholysis (CML) assay. These observations argue for the influence of the, by the Y-chromosome coded, H-Y antigen as a non MHC minor transplantation antigen (5-7). Furthermore, Sparkes et al. (8) reported on the impact of the MNSs blood group antigen system on the occurrence of graft-versus-host disease (GvHD).

In order to determine influences of non HLA-A to DR antigens on the occurrence of GvHD, we investigated the in vitro cytotoxic activity of pre- and post-transplant T lymphocytes of an acute myeloid leukaemia (A.M.L.) patient suffering of a severe GvHD. We evaluated possible influences of the HLA and non HLA antigenic determinants.

In this study, we demonstrate the presence of an apparently HLA-restricted minor histocompatibility antigen which is demonstrable with the CML test with patients' lymphocytes obtained after bone marrow transplantation. The characteristics of this - sofar unknown - target determinant will be discussed.

MATERIAL AND METHODS

The CML assay used has been previously described in detail (9). In this study patients' post-transplant peripheral blood lymphocytes were
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sensitized in vitro for 6 d. with pre-transplant patients' lymphocytes. The effector cells were thereafter tested against randomly selected panel- and family donors as target cells. Cytotoxicity (= the amount of isotope released from the with $^{51}$Cr labeled target cells) was determined and calculated according to the method described earlier (9).

Family "HA" (i.e. patients' family): parents, sibs, donor, patient before and after transplantation were analyzed for all possible polymorphic genetic systems: a) ABO blood groups, including Rhesus, MN, P, S, K, Fy and Lewis; b) complement; c) 13 intracellular enzymes; d) group Five system; e) sex and chromosomal patterns; f) HLA-A,-B,-C,-DR and -SB antigens.

RESULTS AND DISCUSSION

Our study was initiated with the observation that post-transplant lymphocytes from a bone marrow grafted A.M.L. patient (designated "HA") demonstrated strong cytotoxicity against his own pre-transplant lymphocytes and several target cells from the panel (table I). Subsequently experiments were performed, using the above-mentioned effector cells, to analyse the specificity of the cytotoxic target determinant. Table II shows the results of a panel study of randomly chosen individuals tested so far, which revealed a complete association with the HLA-A2 positive target cells. All target cells were lysed which carried the HLA-A2 antigen.

To investigate whether or not this cytotoxic determinant is genetically defined, family studies were performed on the relatives of the positive panel members. The results of the family studies illustrated Mendelian segregation. The cytotoxic determinant segregates in all 10 families tested so far with the HLA-A2 haplotype. In contrast to these observations 2 exceptions have been found in the family of the patient. Absence of cytotoxicity was observed as well on the lymphocytes of the bone marrow donor (see table I) as on the lymphocytes of three haplo-identical siblings.

In search for the identity of the determinant which showed such strong HLA-A2 restriction, we evaluated all possible polymorphic genetic systems which could be responsible for the HLA-A2 restricted lysis. None of these systems seemed to play a decisive role in the observed cytotoxicity.

In conclusion, our data suggest that we may have defined an (to our knowledge first demonstration in man) HLA restricted autosomal minor histocompatibility antigen that might be similar to that described earlier in mice (10). The cytotoxic determinant recognized by patients' post-transplantation cytotoxic lymphocytes appeared after in vivo sensitization (i.e. after bone marrow transplantation). It can be detected in CML on PHA stimulated and unstimulated T cells and on B cell lines (EBV transformed). This example in man of a MHC restricted minor transplantation antigen (designated HA) by which the target determinant would only be detectable if it coexisted with a class I HLA antigen or an antigen which is closely linked to it. The target determinant, which shows a high gene frequency, may be coded for by 1) the HLA region, 2) outside the HLA region but linked to it, or 3) another chromosome.

Since, cytotoxic lymphocyte reactions with this minor histocompatibility antigen appeared after bone marrow transplantation in a patient suffering from severe GvHD, it is obvious to search for its
contribution to the development of GvHR in man. This is especially true, since the relevance of this topic has recently been demonstrated in rodents (11).

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REFERENCES


Table I. Lysis pattern of effector cells of patient "HA" against his own target cells and target cells from unrelated individuals

<table>
<thead>
<tr>
<th>Target cells</th>
<th>HLA phenotypes</th>
<th>% lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1. Patient (pre-transplant)</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>2. &quot; (post-transplant)</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>3. Bone marrow donor</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>4. Unrelated indiv.</td>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td>5. &quot;</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>6. &quot;</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>7. &quot;</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

See materials and methods.

Table II. Possible evidence for an HLA-A2 associated target determinant (panel study)

<table>
<thead>
<tr>
<th>CML</th>
<th>HLA-A2 positive</th>
<th>HLA-A2 negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ 9</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
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