

Exp Hematol 12 (Suppl 15) 77-78 (1984)
© International Society for Experimental Hematology

Detection of Minor Histocompatibility Antigens by MHC Restricted Cytotoxic T Lymphocytes Generated During Graft Versus Host Disease

E. Goulmy¹, E. Blokland¹, J. W. Gratama¹, F. E. Zwaan², and J. J. van Rood¹

¹ Department of Immunohaematology and Blood Bank, University Hospital, Leiden

² Isolation Pavilion, University Hospital, Leiden, The Netherlands

ABSTRACT

We have reported recently the presence of cytotoxic T cells (CTLs) in a bone marrow grafted patient suffering from severe chronic graft versus host disease (GvHD). Analysis of the *in vitro* cytotoxic activity of patients' posttransplant lymphocytes revealed that they detected a non-HLA minor histocompatibility (H) antigen (designated minor HA-1) and that they were MHC restricted. Large scale expansion of these MHC restricted anti-minor H CTLs provided us with large amounts of cellular typing reagents. The aim of this study is three-fold. First: confirmation and extension of our first observation *i.e.*: generation of CTLs with posttransplant lymphocytes of bone marrow grafted patients and its correlation with the occurrence of chronic GvHD. Second: with the use of the *in vitro* generated CTLs, identification of other minor H antigens, *i.e.* obtainment of more anti-minor H cellular typing reagents. Third: with the use of the latter anti-minor H specific CTLs: retrospective typing of HLA identical bone marrow donor and recipient pairs. We studied sofar posttransplant lymphocytes of 13 HLA matched bone marrow recipients with no GvHD (N = 2), acute (N = 7) or chronic (N = 4) GvHD. Strong cytotoxicity was observed with posttransplant lymphocytes from 5 patients (4 of which suffered from chronic GvHD). Analysis of the *in vitro* cytotoxic activity revealed identification of CTLs directed against at least four different minor H antigens. Retrospective typing with the minor H specific CTLs detected sofar only minor H incompatibilities between bone marrow donors and recipients when the recipient was suffering from GvHD.

INTRODUCTION

Graft versus host reactions are mediated by subpopulations of donor T cells and can be attributed to non-MHC antigens (Korngold and Sprent 1978; Kindred 1983). At present no alloimmune sera are available to type for non-HLA antigens which are relevant in the pathogenesis of GvHD. Therefore we aimed at obtaining cellular reagents identifying minor H antigens. *In vitro* priming of lymphocytes

from *in vivo* immunized individuals can lead to recognition of human minor alloantigens as described earlier (Goulmy et al. 1977; Elkins et al. 1982). We used lymphocytes from bone marrow grafted patients in order to determine the role of non-HLA determinants in the induction of GvHD.

The first patient to be studied suffered from Acute Myeloid Leukaemia, received a bone marrow graft from an HLA identical sibling donor, and developed severe chronic GvHD. CTLs could be generated by *in vitro* sensitization of patients' posttransplant lymphocytes against his own pretransplant lymphocytes. We reported earlier (Goulmy et al. 1982, 1983) that with the use of the latter CTLs a minor transplantation antigen (designated minor HA-1) could be identified which was recognized in association with the three self class I HLA antigens. Patients' posttransplant lymphocytes could be expanded and minor HA-1 specific CTLs obtained, frozen and adequately used as typing reagents.

In the study to be reported here 13 patients who received HLA identical bone marrow grafts were investigated for presence of CTLs. In this study we demonstrate that indeed such CTLs can be generated from patients suffering from chronic GvHD and that they recognize at least four different non-HLA determinants. The presence of mismatches for these non-HLA determinants in HLA identical sibling donor-recipient pairs correlated significantly with the occurrence of GvHD.

MATERIALS AND METHODS

The same culture protocol which was successfully used for generation of anti-minor HA-1 CTLs (Goulmy et al. 1982, 1983) was applied. Posttransplant lymphocytes from 13 recipients, of HLA matched bone marrow grafts, were sensitized *in vitro* for 6 days with patients' pretransplant lymphocytes. The effector cells harvested at day 6 were tested in the CML assay for specific cytotoxic activity (Goulmy et al. 1983), as well as expanded in order to obtain cytotoxic T cell lines.

RESULTS AND DISCUSSION

Table 1 shows that with posttransplant lymphocytes from 5 patients, 4 of which suffered from chronic GvHD, cytotoxicity was observed which was directed against their own pretransplant lymphocytes. Analysis of the cytotoxic activity, against a limited panel of healthy unrelated individuals as target cells, revealed identification of at least four different CTL populations directed each against different minor H antigen. The recognition structures (designated minor HA-1, minor HA-2, minor HA-3, and minor HA-4) and the restricting elements necessary for associative recognition are shown in table 1.

Table 1. Detection of cell mediated anti-non HLA activity after bone marrow transplantation.

	CML	
	pos.	neg.
No GvHD	0	2
Acute GvHD	1ψ	6
Chronic GvHD	4φ	0

HLA typing of these patients:

φ1.	A2, A2, B27, Bw62, Cw1,3, DR1,4
ψ2.	A1, A11, B8, B40, Cw3, DR2,3
φ3.	A2, A3, B18, Bw44, Cw7, DR4,6
φ4.	A2, A29, Bw49, Bw44, Cw-, DR5,7
φ5.	A1, A2, B7, Cw7, DR2,3

	Restricting element	Recognition structure
CTLs: 1	A2, B27, Bw62	minor HA-1
2	not yet known	minor HA-2
3	A2	minor HA-3
4	A2	allo HLA-B8
5	A2	minor HA-4
		minor HA-1 (?)

The restricting elements (i.e. HLA-A2, Bw62 and B27) and the non-HLA determinants (i.e. minor HA-1) of the CTLs derived from patient 1 have been extensively described before (Goulmy et al. 1982, 1983).

Another minor H antigen (i.e. minor HA-2) was recognized by CTLs derived from patient 2; in search for the restricting elements, analysis of the cytotoxic activity against a limited number of unrelated target cells identified so far no restriction specificities. Results obtained with anti-minor HA-3 CTLs (patient 3) indicate that they recognize possibly not only an HLA-A2 restricted non-HLA determinant but also a non-self HLA class I alloantigen (i.e. HLA-B8). This might also be an example of "self + x (minor?) equals allo" (Bevan 1977). Analysis of CTLs obtained from patient 4 revealed recognition of other minor H antigens (i.e. minor HA-4) recognized through the HLA-A2 antigen as restricting element. The HLA-A2 restricted CTLs derived from patient 5 recognized a minor H antigen most probably identical to minor HA-1.

The 6 days cytotoxic effector cells could be expanded for longer periods of time. Cytotoxic T cell lines were obtained, frozen and used as typing reagents. These reagents were then used to detect minor H incompatibilities between HLA identical donor and recipient. The results of a series of retrospectively typed

HLA identical bone marrow donor and recipient pairs are shown in table 2.

Table 2. Cellular typing of human minor H antigens with CTLs generated during GvHD.

	identical for minor HA-1 to HA-4	incompatible for 1 or more minor H antigens
do/rec.		
no GvHD	7	0
do/rec.		
acute GvHD	4	1
do/rec.		
chronic GvHD	3	7

As demonstrated above, incompatibilities for minor H antigens between HLA identical bone marrow donor and recipient were so far mainly found in the group of patients suffering from chronic GvHD.

Hopefully, prospective typing for these minor H antigens can mitigate at least one of the obstacles for successful bone marrow transplantation.

REFERENCES.

- Bevan MJ (1977) Killer cells reactive to altered-self antigens can also be allo-reactive. P N A S 74:2094-2098.
- Elkins WL, Pierson G, Negendank W, Zier KS (1982) Recognition of human minor alloantigen(s) by cytotoxic lymphocytes in vitro. Immunogenetics 15:485-499.
- Goulmy E, Termijtelen A, Bradley BA, van Rood JJ (1977) Y-antigen killing by T cells of women is restricted by HLA. Nature 266: 544-545.
- Goulmy E, Gratama J-W, Blokland E, Zwaan FE, van Rood JJ (1982) Recognition of an - as yet unknown - minor transplantation antigen by posttransplant lymphocytes from an AML patient. Exp Hemat 10. 127-129.
- Goulmy E, Gratama J-W, Blokland E, Zwaan FE, van Rood JJ (1983) A minor transplantation antigen detected by MHC-restricted cytotoxic T lymphocytes during graft-versus-host disease. Nature 302:159-161.
- Kindred B (1983) H-2 restricted GvH reaction caused by T cells from normal donors of certain strains. Immunogenetics 17:203-209.
- Korngold R, Sprent J (1978) Lethal graft versus host disease after bone marrow transplantation across minor histocompatibility barriers in mice. Prevention by removing mature T cells from marrow. J Exp Med 148: 1687-1698.

Supported in part by the Dutch Foundation for Medical Research (FUNGO) which is subsidized by the Dutch Organization for the Advancement of Pure Research (ZWO), the J.A. Cohen Institute for Radiopathology and Radiation Protection (IRS).