Reprinted from Nature, Vol 302, No 5904, pp 159 161, 10 March, 1983

A minor transplantation antigen detected by MHC-restricted cytotoxic T lymphocytes during graft-versus-host disease

E. Goulmy, J. W. Gratama, E. Blokland, F. E. Zwaan* & J. J. van Rood

Department of Immunohaematology and Blood Bank, and * Isolation Pavilion, University Hospital Leiden, Rijnsburgerweg 10, 2133 AA Leiden, The Netherlands

Transplantation of bone marrow can give rise to graft-versus-host desease when donor T lymphocytes, mismatched with the host for major histocompatability (MHC) antigens, become sensitized and attack host tissues. However, graft-versus-host desease can also arise between donor and host with compatible MHC antigens but mismatched for a minor histocompatability antigen[1-3]. We report here on the occurrence of severe acute graft-versus-host disease in a male patient with acute myeloid leukaemia who had received bone marrow matched for MHC (HLA) antigens from his sister. Strong cytotoxicity of the post-transplantation (that is, donor) lymphocytes against the patient's pretransplantation lymphocytes was found. Thus, the transplanted lymphocytes differed in a non-HLA antigen from the patient. The possible role of this strong cytotoxic minor histocompatability antigen in the development of graft-versus-host disease in man is being evaluated. Furthermore, with the use of cytotoxic T-cell lines, derived from the patient's 6 day effector cells, we are now able to type for it before grafting.

Following transplantation, karyotype analysis confirmed that all the patient's post-transplantation lymphocytes were of donor origin The initial experiment demonstrated that post-transplantation lymphocytes from the patient (HA) had strong cytotoxic activity, as measured by the cell-mediated lympholysis (CML) assay, against his own pre- but not post-transplantation lymphocytes[4] The patient's cytotoxic effector cells (that is, the patient's post-transplantation peripheral blood lymphocytes sensitized *in vitro* for 6 days with pre-transplantation lymphocytes) used in this initial experiment also lysed two out of four randomly chosen control target cells (Table 1) Lymphocytes of the donor were not lysed

These cytotoxic effector cells were then tested in the CML assay against target lymphocytes from the parents and three siblings (haploidentical to the patient), Fig 1 Besides absence of cytotoxicity against the lymphocytes of the bone marrow donor, absence of lysis was repeatedly demonstrated against the lymphocytes of one out of three HLA-identical siblings which shared one HLA haplotype with the patient As the cytotoxicity pattern in the patient s family showed a difference between HLA-identical siblings, we hypothesized that the target antigen is encoded outside the HLA region but the response to it is restricted by HLA

In an attempt to identify the determinant, we examined the following polymorphic genetic systems in the patient's family which could be a marker for a minor histocompatibility antigen (1) blood groups, including ABO, Rhesus, MN, P, S, K, Fy and Lewis, (2) complement polymorphic markers[5], (3) immunoglobulin allotypic markers GM, AM and KM (ref 6), (4) 13 intracellular enzymes ACP1, ADA, AK1, ALAD, DIA2, ESD, GLO1, GPT1, PGD, PGM1, PGM3, PGP and SOD1 (ref 7), (5) group Five system[8], and (6) sex and chromosomal patterns For none of these systems could we demonstrate a difference between either donor and patient before transplantation or between sibling 3 versus 4 and 5 which correlated with the CML results

To investigate whether the recognition of the minor antigen HA' is HLA-restricted, as is the response to H-Y in HLA-A2-positive female aplastic anaemia patients[9], a random panel

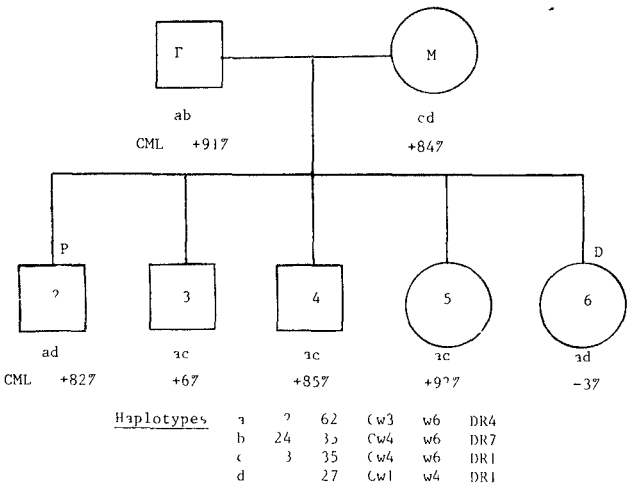

Fig. 1 Pattern of lysis in CML in the patient's family The patient s cytotoxic effector cells (see legend to Table 1) were tested against his family members F, father, M, mother, P (2), patient, D (6), donor, 3, 4 and 5 are siblings haploidentical to the patient The % lysis (which represents the mean value of six experiments) at one effector target cell ratio (50 1) is shown a, b, c, d Delineate parental haplotypes

analysis was performed The cytotoxic effector cells, that is, patient's post-transplantation peripheral blood lymphocytes sensitized *in vitro* with pre-transplantation patient's lymphocytes were tested in the CML assay against more than 150 randomly chosen unrelated healthy individuals The results (Table 2) showed an almost complete association with the HLA-A2 determinant but in addition, lysis of target cells which carried either the HLA-B27 or the HLA-Bw62 antigen was also found All antigens belong to the HLA genotypes of donor and patient, that is, they are 'self' antigens These results suggest that the response to the minor antigen is restricted to these 'self' HLA antigens The panel study also showed that the minor antigen is found at a high frequency in the random population, 95 5% of the HLA-A2-positive, 20% of the HLA-B27-positive and 60% of the HLA-Bw62-positive panel donor cells tested showed significant lysis (Table 2)

The occurrence of an HLA-A2 variant in the patient's family seems unlikely to explain these results because cytotoxic T lymphocytes specific for an HLA-A2 variant[10] did not lyse the patient's cells (pre- and post-transplantation) or those of his family (data not shown) Preliminary biochemical analysis showed no chemical differences between the patient's lymphocytes before and after transplantation (H Ploegh, personal communication)

To further investigate the cytotoxic determinant, family studies were performed by assaying target cells derived from relatives of HLA-A2-positive panel members The cytotoxic determinant segregated in 12 families with the haplotype carrying the HLA-A2 antigen Furthermore, in two HLA A/C cross-over families, the target determinant segregated with the HLA-A2 antigen One example of such a family is shown in Fig 2 Consequently, the minor transplantation antigen which is seen in association with the HLA-A2 antigen shows both a high frequency in the panel and in the family analysis and a codominant inheritance Only five HLA-A2-positive individuals, whose lymphocytes were not lysed, have been found so far, two from the family of the patient (Fig 1) and three HLA-A2-positive panel members (Table 2) Family studies of non-HLA-A2-, but HLA-B27- or HLA-Bw62-positive panel members have still to be performed

An attempt was made to identify whether or not the minor transplantation antigen which is recognized in association with HLA-A2 is distinct from those recognized by HLA-Bw62

Table 1 % Lysis obtained with post transplantation effector cells of patient HA

Target cells	HLA phenotypes A		B		C	DR	SB	% Lysis
Patient (pre transplantation)	2		27	62	1	1 4	4 4	+59
Patient (post-transplantation)	2		27	62	1	1 4	4 4	−1
Bone marrow donor	2		27	62	1	1 4	4 4	−3
Unrelated individual	3	31	27	40	2 3	4		+5
Unrelated individual	1	32	8	44		3 7		−7
Unrelated individual	2	11	51	62	3	1 2		+26
Unrelated individual	2		7	27	2	2 6		+35

The effector cells (phenotype HLA-A2, A2, B27, Bw62, Cw1, Cw3, DR1, DR4, SB4) used throughout this study were patient's post-transplantation peripheral blood lymphocytes (that is donor cells) sensitized *in vitro* for 6 days with pre-transplantation patient s lymphocytes These effector cells were tested against randomly selected panel and family donors (see also Table 2, Figs 1, 2) as target cells The CML assay used has been previously described in detail Cytotoxicity in Tables 1–3 and Figs 1, 2 the amount of isotope released from the (^{51}Cr-labelled target cells) was determined and calculated according to methods described earlier[16] All experiments were repeated at least twice at six effector to target ratios Lysis at only one effector target ratio (50 1, that is, 2.5×10^5 5×10^3) is shown in this table Positive and negative assignment was made on the basis of a 10% specific ^{51}Cr release value Standard errors of the means of triplicates were always less than 5% No lysis occurred when responder cells were tested against the relevant target cells after the following cultures *a*, patient s post transplantation lymphocytes as responder cells with the donor cells as stimulator cells, *b*, donor cells as responder cells with patient's post-transplantation lymphocytes as stimulator cells, and *c*, donor cells as responder cells with the patient s pre transplantation lymphocytes as stimulator cells

and/or HLA-B27 Cold target cell inhibition studies (Table 3) and the T-cell clone analyses (data not shown) showed unequivocally that the HLA-A2-, B27- and Bw62-restricted T-cell subsets were all separate Nevertheless, we have as yet no data to indicate whether or not the non-HLA antigen which is seen in association with HLA–A2 is the same as those seen in association with HLA-B27 and HLA-Bw62

Our data suggest that we have defined an HLA-restricted minor histocompatibility antigen(s) that might be similar to that described earlier in mice[2,3] We have called this antigen HA The restricting elements were the HLA-A and the HLA-B antigens of the patient's lymphocytes The non-HLA determinant remains unidentified at present, but we assume that the parents are both heterozygous for this antigen with a high gene frequency, the bone marrow donor and sibling 3 (Fig 1) being homozygous for the allele 'HA' which has a gene frequency of ±0 07 if HLA-A2 is present

Graft-versus-host reactions are mediated by subpopulations of donor T cells and can be attributed to non-MHC antigens For example, peripheral blood lymphocytes of two female aplastic anaemia patients showed HLA-restricted H–Y killing in a CML assay These observations argue for the influence of the male H–Y antigen as a non-MHC minor transplantation antigen[9,11,12] In agreement with this, sex-mismatched bone marrow grafts do less well than sex-matched grafts especially if the donor is a female[13] The possible role of the MNSs blood group antigen system on the occurrence of graft-versus-host disease has been reported by Sparkes *et al*[14] Elkins *et al*[15] described the recognition of a human minor alloantigen after *in vitro* stimulation with allogeneic cells of lymphocytes derived from a multi-transfused patient

We believe that our findings might be of importance in the understanding of the biological role of non-HLA transplanta-

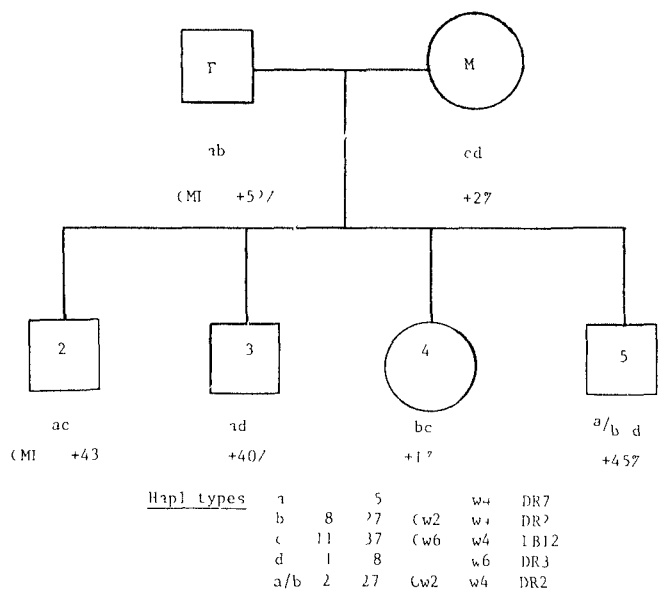

Fig. 2 Segregation pattern of the HLA-restricted minor histocompatibility antigen in an HLA A/C cross-over family The % lysis at only one effector target cell ratio (50 1) is shown

Table 2 Analysis of HLA restricted anti-HA antigen lysis

	Target cells Serological typing for HLA-A2$^+$, B27$^-$, Bw62$^-$	HLA-A2 , B27$^+$, Bw62$^-$	HLA-A2 , B27 , Bw62$^+$	HLA-A2 , B27$^-$, Bw62$^-$
CML $^+$	72	2	11	0
CML $^-$	3	13	6	47

The level of lysis obtained against the HLA-B27- and HLA Bw62-positive target cells was always lower than that obtained against HLA-A2 positive target cells Target cells carrying more than one restricting element are not included in this table

Table 3 Competitive inhibition experiments

^{51}Cr labelled target cells	Cold inhibitors added	% Lysis
HLA A2 , Bw62 B27	None	+99
	HLA A2$^+$, Bw62 , B27	+39
	HLA A2 , Bw62$^+$, B27	+99
HLA A2$^+$, Bw62 , B27	None	+97
	HLA A2$^+$, Bw62 , B27	+26
	HLA A2 , Bw62 , B27$^+$	+95
HLA A2 , Bw62$^+$ B27	None	+58
	HLA A2 , Bw62$^+$ B27	+19
	HLA A2$^+$, Bw62 , B27	+55
	HLA A2 , Bw62 B27$^+$	+54

Patient s cytotoxic effector cells (see legend to Table 1) were tested against target cells that were positive for only one of the restricting HLA antigens but which were all positive for the minor antigen HA The inhibitory capacity of non labelled target cells carrying only one of the restricting elements plus the minor antigen was measured The ratio labelled unlabelled target cells was 10 1 The % lysis of effector target ratio of 50 1 is shown The competitive inhibition protocol has been described in detail[16]

tion systems and in its management in organ transplantation. The fact that the cytotoxic T lymphocytes described here were found in a patient suffering from severe acute graft-versus-host disease suggests that incompatibility for the HA antigen might have had a role in the induction of the disorder. At present, however, it is possible, using cytotoxic T-cell lines derived from patient's 6-day effector cells, to type for the strong cytotoxic minor histocompatibility antigen HA before grafting. Serotyping for HLA with alloimmune sera (together with mixed lymphocyte culture between donor and recipient) appears to be insufficient to determine all the cell interactions dependent on HLA, and CML typing with cytotoxic T lymphocytes induced after *in vivo* priming might provide important information.

We thank Mrs A. Pesant for typing the manuscript, Ms T. Irti and Ms T. Goekoop for technical assistance, family Hartog for generous blood donations, Dr P. Meera Khan, Dr G. Hauptmann and Ms G de Lange for help with assays, and Dr A. Munro for critical reading of the manuscript. This work was 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).

Received 4 November 1982, accepted 5 January 1983

1 Bevan, M J *J exp Med* **142,** 1349–1364 (1975)
2 Korngold, R & Sprent, J *J exp Med* **148,** 1687–1698 (1978)
3 Hamilton, B L, Bevan, M J & Parkman, R *J Immun* **126,** 621–625 (1981)
4 Goulmy, E, Gratama, J W, Blokland, E, Zwaan, F E & van Rood, J J *Expl Haemat* **10,** Suppl 10, 127–129 (1982)
5 Hauptmann, G *Blood Transfusion Immunohaemat* **22,** 587–614 (1979)
6 van Loghem, E in *Handbook of Experimental Immunology* Vol 1 (ed Weir, D M) 11 1–11 6 (Blackwell, Oxford, 1978)
7 Meera Khan, P, Rijken, H, Wijnen, J Th, Wijnen, L M M & De Boer, L F M in *Perspectives in Vertebrates Science* Vol 2 (ed de Boer, L E M) 61–108 (Junk, The Hague, 1982)
8 van Leeuwen, A, Eernisse, J G & van Rood, J J *Vox Sang* **9,** 431–446 (1964)
9 Goulmy, E, Termijtelen, A, Bradley, B A & van Rood, J J *Nature* **266,** 544–545 (1977)
10 Horai, S, Poel, J v d & Goulmy, E *Immunogenetics* **16,** 135–142 (1982)
11 Goulmy, E, Bradley, B A, Lansbergen, Q & van Rood, J J *Transplantation* **25,** 315–319 (1978)
12 Goulmy, E, Hamilton, J D & Bradley, B A *J exp Med* **149,** 545–555 (1979)
13 Zwaan, F E & Hermans, J *Expl Haemat* **10,** Suppl 10, 64–69 (1982)
14 Sparkes, R S *et al Tissue Antigens* **15,** 212–215 (1980)
15 Elkins, W L, Pierson, G, Negendank, W & Zier, K S *Immunogenetics* **15,** 485–499 (1982)
16 Goulmy, E in *HLA Typing Methodology and Clinical Aspects* Vol 2 (eds Ferrone, S & Solheim, B) 105–122 (CRC, New York, 1982)