Minor Histocompatibility Antigen H-Y Is Expressed on Human Hematopoietic Progenitor Cells

Paul J. Voogt, Els Goulmy, Willem E. Fibbe, Willemien F. J. Veenhof, Anneke Brand, and J. H. Frederik Falkenburg
Laboratory of Experimental Hematology, Department of Hematology, and Department of Immunohematology and Blood Bank, University Medical Center, 2333 AA Leiden, The Netherlands

Abstract

Polymorphic minor transplantation antigens probably play an important role in immune mediated graft rejections of bone marrow transplants. Mapping of these antigens on hematopoietic progenitor cells (HPC) is important since these antigenic determinants may serve as target structures in the rejection process, and it ultimately opens the possibility to match for these antigens. Using a cell-mediated cytotoxicity assay with H-Y-specific cytotoxic T lymphocytes as effector cells, a dose-dependent growth inhibition up to 100% of myeloid (CFU-GM), erythroid (BFU-E) and multipotential (CFU-GEMM) HPC of male donors was obtained, indicating expression of the H-Y antigen on these progenitor cells. In contrast, inhibition of relatively mature erythroid and myeloid progenitor cells was only 40–50%, indicating that the recognition of the H-Y antigen diminished during maturation of erythroid and myeloid HPC. Our results show that the H-Y antigen can be recognized on HPC as a target for cytotoxic T cell responses. This may be important in graft rejection of male donor bone marrow grafts by female recipients.

Introduction

Allogeneic bone marrow transplantation (BMT)[1] is being successfully used for the treatment of various hematological disorders, such as aplastic anemia (1, 2) and leukemia (3, 4). However, graft-versus-host disease (GVHD) (5, 6) and graft failure (7, 8) are major obstacles to successful transplantation, causing serious morbidity and mortality. There is a particularly high incidence of graft rejection in recipients of HLA-non-identical grafts (9, 10). This is in accordance with the finding that the polymorphic major histocompatibility class I and class II antigens are expressed on hematopoietic progenitor cells (HPC), as shown previously (11–13). However, graft failures have also been observed in recipients of HLA-identical transplants, particularly in aplastic anemia patients who had been extensively transfused before transplantation (2).

Recently, T lymphocyte depletion of the marrow graft, as a method to prevent GVHD, has been found to be associated with an increase in the incidence of graft failures in HLA identical transplants (14–17). Since the incidence can be reduced by a more intensive pretransplant immunosuppressive treatment of the recipient (18–20), it is likely that an immune-mediated rejection is involved in these cases. It has been demonstrated that immunocompetent lymphocytes can survive the conditioning regimen for BMT (21, 22). Therefore, graft rejections are probably mediated by radioresistant host lymphocytes that recognize polymorphic antigenic determinants other than HLA antigens on donor marrow cells. Accordingly, mapping of these minor histocompatibility antigens on HPC is of major importance.

The minor histocompatibility antigen H-Y is coded for by the Y chromosome and therefore is expressed on cells of male individuals (23). It was discovered as a transplantation antigen by Eichwald and Slimser in 1955, who found that skin grafts from male donors could be rejected by female recipients from certain strains of inbred mice (24). In 1977, Goulmy et al. showed the HLA class I restricted recognition of the H-Y antigen in humans (25). The human H-Y antigen can only be studied in vitro using cellular techniques such as cell-mediated cytotoxicity. Minor transplantation antigens, such as H-Y, characteristically provoke good T cell responses but poor antibody responses. Although antibody responses to H-Y antigen have been described in mice, titers are low while attempts to make high-titered monoclonal antibodies to H-Y have been unsuccessful. Furthermore, antibody responses to H-Y do not correlate well with graft rejection in mice, in contrast to T cell responses (26).

In this study we investigated, using a cell-mediated cytotoxicity assay, the expression of the H-Y antigen on mature and immature human hematopoietic progenitor cells as well as on mature peripheral blood cells of both male and female donors. We demonstrate that the H-Y antigen is expressed on all male immature hematopoietic progenitor cells (CFU-GEMM, CFU-GM, BFU-E), and that expression diminishes during erythroid and myeloid differentiation.

Methods

Establishment of the anti-H-Y CTL line

The anti-H-Y CTL line was established as described previously (25, 27). Briefly, peripheral blood mononuclear cells (MNC) were isolated from a multitransfused female aplastic anemia patient. These cells were used as responder cells in a standard mixed lymphocyte culture:

Address reprint requests to Dr. Voogt, Department of Immunohematology and Blood Bank, Bldg. 1, E3-Q, University Medical Center, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands.

Received for publication 16 June 1987 and in revised form 29 January 1988.

1. *Abbreviations used in this paper:* BFU-E, burst-forming unit, erythrocytes; BMT, bone marrow transplantation; CD, cluster of differentiation; CFC, cluster-forming cell; CFU-E, CFU erythrocytes CFU-GM, colony-forming unit granulocytes, macrophages; CFU-GEMM, CFU granulocytes, erythrocytes, macrophages, megakaryocytes; CTL, cytotoxic T lymphocyte; GVHD, graft-versus-host disease; HPC, hematopoietic progenitor cells; α-MEM, α-modified Eagle's minimal essential medium; MNC, mononuclear cell; TCGF, T cell growth factor.

J. Clin. Invest.

0021-9738/88/09/0906/07 $2.00
Volume 82, September 1988, 906–912

10^7 responder cells were incubated with 10^7 irradiated MNC from an HLA-matched unrelated male donor, in a medium consisting of 20 ml Hepes-buffered RPMI 1640 with 15% prescreened pooled human AB serum supplemented with 0,1% gentamycin and 10 mM/liter L-glutamin (RPMI plus 15% serum). The cells were cultured for 6 d at 37°C in a fully humidified atmosphere of 5% CO_2. After 6 d the effector cells were further expanded by weekly restimulating 10^5 cells with 10^6 MNC of the original male donor in 1 ml medium consisting of 20% TCGF (T cell growth factor; Biotest, Offenbach, West Germany) in RPMI plus 15% serum. In this way cytotoxic T cell (CTL) lines were grown, and then cryopreserved in liquid nitrogen. Before use, the CTL line was thawed for 1 min in a 37°C waterbath, diluted in RPMI plus 50% serum, washed once in the same medium, and further expanded for 3–5 d at a concentration of 10^5 cells/ml in 20% TCGF in RPMI plus 15% serum. The cytotoxic H-Y specific activity was tested in a standard ^{51}Cr-release assay (28), using PHA-blasts as target cells. Surface marker analysis of the CTL line was performed using an indirect immunofluorescence technique with murine monoclonal antibodies and a fluorescence-activated cell sorter (FACS analyzer; Becton-Dickinson Immunocytometry Systems, Mountain View, CA) (29). The expression of antigenic determinants on the effector cells was studied with monoclonal antibodies against the T lymphocyte markers CD3 (OKT3; Ortho Diagnostic Systems, Raritan, NJ), CD4 (Leu3a) and CD8 (Leu2a; both from Becton-Dickinson Monoclonal Center Inc., Mountain View, CA), the B-cell markers CD19 (Leu12; Becton-Dickinson) and CD20 (B1; Coulter Clone, Coulter Immunology, Hialeah, FL), HLA-DR (Becton-Dickinson), and using a monoclonal antibody recognizing CD25, the interleukin 2 receptor (TAC, Becton-Dickinson).

Collection of bone marrow

Normal human bone marrow of donors for BMT was obtained, after informed consent, by aspiration from the posterior iliac crests. The cells were collected in HBSS with 100 U/ml preservative-free heparin. Bone marrow mononuclear cells were isolated by centrifugation (1,000 *g*, 30 min, 20°C) over Ficoll-Isopaque (1.077 g/cm^3). In some experiments freshly obtained bone marrow cells were used, in others, bone marrow cells were first cryopreserved in liquid nitrogen, as described previously (30). Immediately before use the cells were thawed for 1 min in a 37°C waterbath, diluted in Hepes-buffered RPMI plus 20% FBS at 0°C, washed once in the same medium, and then washed again in RPMI plus 15% serum. The cells were resuspended in RPMI plus 15% serum at a concentration of 5×10^5 viable cells/ml.

Cell-mediated cytotoxicity assay

The cell-mediated cytotoxicity assay was performed as described previously (31). Briefly, a quantity of 1.25×10^5 bone marrow cells in 0.25 ml RPMI plus 15% serum was mixed with an equal volume of this medium containing cytotoxic T lymphocytes (CTLs). The effector/target cell ratios varied from 1:2 to 4:1. The cell mixture was centrifuged (1,000 *g*, 15 s) to establish cell-cell contact between CTLs and bone marrow cells, and then incubated for 4 h at 37°C in a fully humidified atmosphere of 5% CO_2. After incubation the cells were washed once in RPMI plus 15% serum, resuspended in α-modified Eagle's minimal essential medium (α-MEM; Flow Laboratories, Irvine, CA), and subsequently cultured for CFU-GM, CFU-E/BFU-E, and CFU-GEMM. As a control to establish the necessity of cell-cell contact between CTLs and bone marrow cells, and to exclude the possibility of nonspecific inhibition of HPC growth due to the presence of cytotoxic cells in the semisolid culture medium, CTLs were added to the bone marrow cells immediately before plating. All CTLs were irradiated (20 Gy) before use to prevent colony formation by these cells.

CFU-GM

A quantity of 10^5 bone marrow cells was cultured in 1 ml medium containing 20% FBS (Rehatuin, Kankakee, IL), 20% leukocyte-conditioned medium (32), 20% α-MEM and 40% methylcellulose 2,25% in a fully humidified atmosphere of 5% CO_2 at 37°C in 35 mm plastic dishes. CFU-GM colonies, defined as granulocytic, monocytic or eosinophilic aggregates of more than 20 cells, were scored under an inverted microscope on day 10. In some experiments both immature and mature myeloid progenitor cells were scored sequentially. In these cases 10^5 fresh bone marrow cells were cultured as described above. After 4 d of culture the number of clusters of 5–20 cells were counted, determining the cluster forming cells day 4 (CFC day 4) (33). After 7 and 10 days the number of CFU-GM colonies were scored.

CFU-E/BFU-E

A quantity of 10^5 bone marrow cells was cultured in 1 ml medium containing 20% FBS, 20% leukocyte-conditioned medium, 5% 10^{-3} M 2-mercaptoethanol, 5% Iscove's modified Dulbecco's medium, 5% deionized bovine serum albumin (Sigma Chemical Co., St. Louis, MO), 5% human transferrin, and 40% methylcellulose 2.25% with 1 U/ml erythropoietin (step III; Connaught Laboratories, Toronto, Canada) in 35 mm plastic dishes, in a fully humidified atmosphere of 5% CO_2 at 37°C. CFU-E, defined as clusters of 8-64 hemoglobinized cells, were scored on day 7. The number of BFU-E was scored on day 14.

CFU-GEMM

A quantity of 10^5 bone marrow cells was cultured in 1 ml medium containing 30% ABO-compatible human heparin plasma, 7.5% phytohemagglutinin-leukocyte-conditioned medium (34), 5% 10^{-3} M 2-mercaptoethanol, 5% deionized BSA, 5% human transferrin, 5% Iscove's modified Dulbecco's medium and 40% methylcellulose, 2.8% with 1 U/ml erythropoietin (2,5%) in 35 mm plastic dishes, in a fully humidified atmosphere of 5% CO_2 at 37°C. CFU-GEMM, defined as colonies containing at least both erythroid and myeloid cells (35), were scored on days 14–18.

Normal values and calculations of HPC growth

100% growth was defined as the number of colonies cultured from 10^5 untreated bone marrow mononuclear cells. Normal values of HPC growth in our laboratory are 269±25 for CFC day 4, 82±7 for CFU-GM day 7, 182±15 for CFU-GM day 10, 121±12 for BFU-E, 149±6 for CFU-E and 16±1 for CFU-GEMM (mean±SE). In cellular cytotoxicity assays the percentages of surviving HPC were calculated by dividing the total number of colonies by the number of colonies in the untreated control cultures.

Isolation of peripheral blood cells

Granulocytes. 2.5 ml peripheral blood was diluted with 7.5 ml RPMI 1640 plus 5% FBS and then mixed with 0.4 ml methylcellulose 2.25%, and allowed to sediment for 30 min. The supernatant was harvested, diluted in RPMI 1640 plus 5% FBS, and centrifuged over Ficoll-Isopaque. The sediment containing the granulocytes was then harvested, and incubated in an NH_4CL solution (10 min, 0°C) to lyse all residual erythrocytes.

Monocytes. After separation of peripheral blood over Ficoll-Isopaque, T cells were removed by rosetting with 2-aminoethylisothiouronium bromide (AET)-pretreated sheep red blood cells (SRBC), and subsequently centrifuging the cell suspension over Ficoll-Isopaque (36). The interphase was harvested and further enriched for monocytes by centrifugation over a Percoll gradient (1.063 g/cm^3) (37).

T, B, and non-B/non-T lymphocytes. Peripheral blood was depleted of monocytes by incubation with carbonyl-iron particles (45 min, 37°C), and subsequent centrifugation over Ficoll-Isopaque (38). The interphase cells were collected, and incubated with AET-pretreated SRBC, and again separated over Ficoll-Isopaque. B and non-B/non-T lymphocytes in the interphase, and the sediment containing the T lymphocytes were harvested separately, and incubated in an NH_4CL solution (10 min, 0°C) to lyse remaining SRBC. The interphase cells were then separated in a B- and a non-B/non-T lymphocyte fraction, using FITC-labeled (FAB_2) anti human Ig antibodies (Nordic Immu-

Table I. Characterization of Anti-H-Y Cytotoxic T Lymphocyte Line

Female responder HLA phenotype	Male stimulator HLA phenotype	Cytotoxic reactivity on PHA blasts			Phenotype	
		Sex	HLA phenotype	% lysis in CML*	Marker	% + cells
A2, A28	A2	M	A2+ B7−	80±6‡	CD3	98
B7, Bw62, Bw6	B7, Bw62, Bw6	F	A2+ B7−	8±3	CD4	13
Cw3	Cw3, Cw7	M	A2− B7+	89±5	CD8	90
DR1, DR2	DR2	F	A2− B7+	7±4	CD19	9
		M	A2− B7−	6±2	CD20	3
					CD25	23
					HLA-DR	97

* E/T cell ratio 20:1. ‡ Mean±SE of four experiments.

nological Laboratories, Tilburg, The Netherlands) and fluorescence-activated cell sorting (FACS IV) (12).

PHA-blasts. Peripheral blood was separated over Ficoll-Isopaque, and the interphase was harvested. 10^7 interphase cells were then cultured in RPMI 1640 plus 15% human AB-serum with 0,1% phytohemagglutinin for 3–5 d.

Purity of the peripheral blood cell preparations

All preparations were stained according to the Wright-Giemsa method, and then scored visually under a microscope. The number of T cells was determined by counting the number of E-rosetting cells. The number of B cells was determined by counting the number of CD-20 positive cells, using a direct immunofluorescence technique. Non-B/non-T lymphocytes were defined as cells with a lymphoid appearance without T or B cell characteristics.

^{51}Cr-release assays

Standard ^{51}Cr-release assays were performed as described previously (28). Briefly, target cells were labeled with 100 μCi $Na_2{}^{51}CrO_4$ for 1 h in a 37°C waterbath, washed three times with HBSS and then resuspended in RPMI plus 15% serum at a concentration of 5×10^4 viable cells/ml. A quantity of 0.1 ml of the effector cell suspension and 0.1 ml of the target cell suspension were added to each well of a round bottomed microtiter plate at E/T ratios ranging from 40:1 to 1:1. To measure spontaneous release of ^{51}Cr, 0.1 ml of the target cell suspension was added to 0.1 ml RPMI plus 15% serum, without effector cells, while maximum release was determined by adding 0.1 ml of the target cell suspension to 0.1 ml of a Zaponine solution. Percentage lysis was determined as follows: experimental mean cpm − spontaneous release cpm/maximum release cpm − spontaneous release cpm × 100. Cold target inhibition assays were performed by adding non-^{51}Cr-labeled (cold) target cells to a specific combination of effector cells and ^{51}Cr labeled (hot) target cells. Percentage inhibition of lysis of hot targets by cold targets was measured as follows: % lysis of hot targets only − % lysis of hot and cold targets/% lysis of hot targets only × 100.

Results

Characterization of the anti H-Y CTL line. Cytotoxic reactivity, specificity and surface marker analysis of the anti H-Y CTL line are shown in Table I. In the ^{51}Cr-release assay, at an E/T cell ratio of 20/1 the CTLs caused 80–90% lysis of PHA blasts from HLA-A2 or -B7 positive male donors, and showed no reactivity against PHA blasts from HLA-A2 or -B7 positive female donors (mean lysis: 7–8%). Because H-Y antigen recognition by these cytotoxic T lymphocytes is restricted by HLA-A2 or -B7 antigens (25) there is no reactivity against PHA blasts from HLA-A2 and -B7 negative male donors (mean lysis: 6%). Surface markers analysis of the anti H-Y CTL line by indirect immunofluorescence showed that 98% of the cells were activated T lymphocytes; the majority (90%) having a cytotoxic/suppressor phenotype.

Reactivity of the anti H-Y CTL line with HPC. When the anti H-Y CTL line was incubated for 4 h with bone marrow mononuclear cells, a dose-dependent inhibition of the growth of CFU-GM, BFU-E and CFU-GEMM from HLA-A2 or -B7 positive male donors was found at E/T ratios varying from 1:2 to 4:1 (Fig. 1). At E/T ratio 4:1 virtually no growth was observed: 6±2% (mean±SE) growth of CFU-GM, 7±5% growth of BFU-E and 4±2% growth of CFU-GEMM, as compared to the untreated control cultures, indicating that the H-Y antigen is expressed on these HPC. There was only partial inhibition of the growth of CFU-E (60±15% growth at the highest E/T ratio). When bone marrow cells from HLA-A2 or -B7 positive female donors were tested, growth of CFU-GM, CFU-E, BFU-E, and CFU-GEMM was not inhibited at the effector/target cell ratios used (colony growth at E/T ratio 4:1: 102±4%, 95±8%, 114±13%, and 85±17%, respectively).

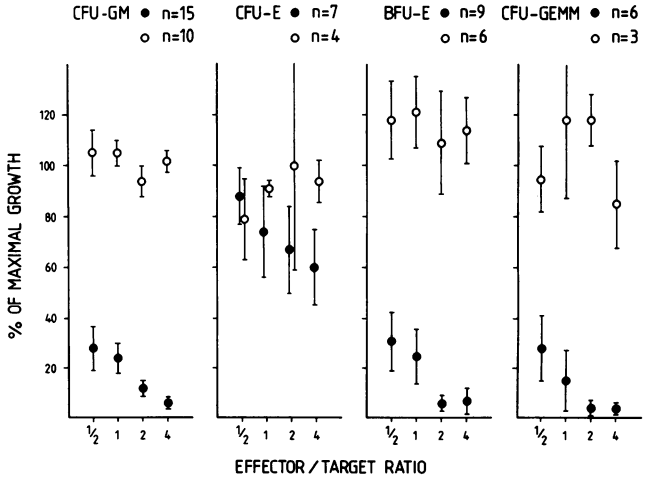

Figure 1. Mean growth of CFU-GM, CFU-E, BFU-E and CFU-GEMM after incubation with the anti-H-Y-CTL line at various E/T cell ratios. Closed symbols represent HLA-A2 or -B7 positive male donor bone marrow cells; open symbols, HLA-A2 or -B7 positive female donor bone marrow cells. Maximal growth is defined by the number of colonies in the untreated control samples. Vertical bars indicate SE.

Table II. Growth of Human Myeloid Hematopoietic Progenitor Cells after Incubation with the Anti-H-Y Cytotoxic T Lymphocyte Line

	Males	Females
CFC day 4	45±3	119±3
CFU-GM day 7	14±5	112±12
CFU-GM day 10	6±6	109±21

Data are expressed as percentage (mean±SE of three experiments) of maximal growth in untreated control cultures. All bone marrow donors were HLA-A2− or -B7 positive.
E/T cell ratio 4:1.

The expression of the H-Y antigen on immature myeloid progenitor cells (CFU-GM day 10 and day 7) was compared with the more mature myeloid HPC (CFC day 4) of male bone marrow donors. As shown in Table II, inhibition of myeloid HPC growth by the anti-H-Y CTL line diminishes during maturation of the progenitors, since there was only 6±6% growth of CFU-GM day 10 and 14±5% growth of CFU-GM day 7 as compared to 45±3% growth of CFC day 4. Control cultures in which female bone marrow cells were used as targets did not show inhibition of growth by the anti-H-Y CTL line.

To exclude the possibility that the observed inhibition was nonspecific, CTLs and bone marrow cells were plated without preincubation. Table III shows that addition of the CTLs to the bone marrow culture at an effector/target cell ratio of 4:1, without incubation before plating, did not result in a significant inhibition of hematopoietic progenitor cell growth, indicating that intimate effector/target cell contact was required for the elimination of the HPC.

The lysis of the HPC by the anti H-Y CTL line was HLA class I restricted: Fig. 2 shows that normal growth of CFU-GM was observed (115±10%) when bone marrow cells from HLA-A2 and -B7 negative male donors were incubated with the anti H-Y CTL line at an effector/target cell ratio of 4:1. To investigate whether the H-Y antigen can also be recognized on HPC in conjunction with other restriction elements we performed some experiments using a recently isolated HLA-A1 restricted anti H-Y cytotoxic T lymphocyte line. Extended population studies showed that this CTL line has characteristics similar to the HLA A2/B7 restricted cell line, except that only cells from HLA-A1 positive male donors are lysed (data not shown). As shown in Table IV, this CTL line lysed HLA-A1 positive male donor HPC but did not inhibit HLA-A1 positive female donor HPC.

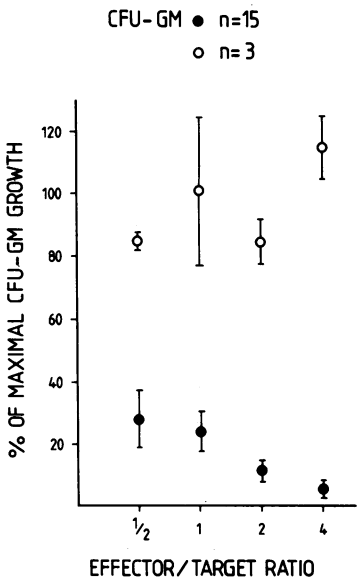

Figure 2. Mean growth of CFU-GM after incubation with the anti-HY-CTL line at various E/T cell ratios. Closed symbols represent HLA-A2 or -B7 positive male donor bone marrow cells; open symbols represent HLA-A2 and -B7 negative male donor bone marrow cells. Maximal growth is defined by the number of colonies in the untreated control samples. Vertical bars indicate SE.

Finally, the expression of the H-Y antigen on mature peripheral blood cells was studied (Table V). We could not demonstrate any lysis of mature granulocytes and erythrocytes of HLA-A2 or -B7 positive male donors by the anti-H-Y CTL line. However, when granulocytes were used as competitors in a cold target inhibition assay, they were able to induce some inhibition of lysis of hot targets, although it was less than inhibition of lysis by cold T lymphocytes (Table VI). This indicates that the H-Y antigen is to some extent expressed on mature granulocytes. The H-Y antigen was found to be clearly expressed on monocytes, T, B, and non-B/non-T lymphocytes.

Discussion

In allogeneic bone marrow transplantation, residual T lymphocytes or other immune cells that have survived chemotherapy and irradiation may recognize certain polymorphic antigenic determinants on HPC of the donor, leading to an immune-mediated rejection of the bone marrow graft (7, 8, 14). Identification of the polymorphic antigens that could serve as target structures in graft rejection ultimately opens the possibility to match for these histocompatibility antigens.

Table III. Growth of Human Hematopoietic Progenitor Cells in the Presence of H-Y-specific CTLs with or without Incubation before Culture

	Incubation with CTLs	CTLs added to culture medium	Number of experiments
CFU-GM	6±2	83±7	13
CFU-E	60±15	93±11	7
BFU-E	7±5	78±7	7
CFU-GEMM	4±2	80±17	5

Data are expressed as percentage (mean±SE) of maximal colony growth in untreated control cultures. In all experiments HLA-A2 or -B7 positive male donors were used. E/T cell ratio 4:1.

Table IV. Growth of Hematopoietic Progenitor Cells after Incubation of HLA-A1 Positive Bone Marrow Donors with the HLA-A1 Restricted Anti-H-Y CTL Line

	Male	Female
CFU-GM	6%*	102%*
CFU-E	40%	97%
BFU-E	12%	114%
CFU-GEMM	0%	100%

* Percent growth as compared to untreated controls.
E/T ratio 4:1.

Table V. Lysis of Peripheral Blood Cells by Anti-H-Y Cytotoxic T Lymphocyte Line

Cell type	Males	Females	Purity of cell population*
			%
Granulocytes	2±2‡	0±1‡	86
Monocytes	46±12	4±0	95
T lymphocytes	50±10	2±2	91
B lymphocytes	40±12	2±1	97
Non-B/non-T lymphocytes	41±7	3±2	98
Erythrocytes	2±1	0±1	99
PHA blasts	69±9	6±2	—

* By Wright-Giemsa staining, E rosetting or presence of surface immunoglobulins.
‡ % lysis in ^{51}CR-release assay (mean±SE of three experiments). E/T cell ratio 40:1.

Storb et al. (8, 39) previously reported that male sex of the donor is associated with a greater risk of graft rejection after allogeneic BMT in patients with aplastic anemia. More recently, Kernan et al. (40) identified male donor sex as a risk factor for graft-failure following T cell-depleted bone marrow transplants. Since graft failure after BMT may often be due to the recognition of unshared polymorphic antigens by the host, the rejection of transplants from male donors by female recipients may be due to recognition of the male-specific minor histocompatibility antigen H-Y on male donor bone marrow cells. In fact, following the rapid rejection by a female recipient of a bone marrow graft from her HLA-identical male sibling, it has been possible to elicit an HLA-restricted H-Y specific cytotoxic T cell response from the peripheral blood of that patient in mixed lymphocyte cultures (25). However, it has not been demonstrated so far that the H-Y antigen is expressed on hematopoietic progenitor cells, and can serve as a target structure for cytotoxic T cell responses.

Our results show that the H-Y antigen is expressed on CFU-GM, BFU-E, and CFU-GEMM (Fig. 1), and that the cell-mediated cytotoxicity of the CTL line directed against this antigen on HPC, is HLA class I restricted (Table I, Fig. 2). H-Y antigen expression of HPC was shown using two anti H-Y CTL lines, with different restricting HLA-antigens, demonstrating that recognition of the H-Y antigen can take place in the context of various HLA-antigens. The effector mechanism by which recognition of the H-Y antigen on HPC of male donors resulted in growth inhibition of HPC, appeared to be clearly dependent on cell-cell contact, since it was not possible to induce growth inhibition without preincubation of CTLs and bone marrow cells before plating (Table III). Therefore, it is unlikely that this effect is due to release of growth inhibitory factors in the medium by the cytotoxic cells.

Table VI. Analysis of Expression of the H-Y Antigen on Granulocytes and T Lymphocytes by Cold Target Inhibition

Cold targets	Percentage inhibition*
Male T lymphocytes	42±6*
Female T lymphocytes	8±3
Male granulocytes	20±5
Female granulocytes	1±3

* Percentage inhibition (mean±SE) of lysis of male donor PHA-blasts, as determined in two triplicate experiments. Effector:hot target:cold target ratios = 40:1:40.

With regard to CFU-E, the recognition of the H-Y antigen by the anti H-Y CTL line was less clear. This may indicate that the expression of the H-Y antigen diminishes during maturation from early (BFU-E) to late (CFU-E) erythroid progenitor cells. The absence of lysis of mature peripheral blood erythrocytes was expected since these cells do not express HLA class I antigens (41), and therefore cannot be recognized by the CTL line.

A similar decrease in recognition of the H-Y antigen was also observed during myeloid differentiation: whereas there was maximal inhibition of growth of the early myeloid progenitors CFU-GM day 10, there was only partial inhibition of growth of late myeloid progenitors (CFC day 4), and no obvious recognition of the H-Y antigen on mature granulocytes in standard ^{51}Cr-release assays (Table V). On the other hand cold target inhibition studies indicated that the H-Y antigen is expressed to some extend on mature granulocytes (Table VI). Several factors may contribute to the diminished recognition of the H-Y antigen during myeloid differentiation. Besides a decreased expression of the H-Y antigen, we cannot exclude that this decrease in growth inhibition is partly due to a diminished expression of the HLA class I determinants, since there is evidence that the expression of HLA class I antigens diminishes during myeloid differentiation (42, 43) although they are clearly present on mature granulocytes (41, 43). Alternatively, it may be that more mature myeloid cells are simply less sensitive targets for cell mediated lysis. The H-Y antigen was clearly expressed on other mature peripheral blood cells, since monocytes, T, B and non-B/non-T lymphocytes were effectively lysed by the anti H-Y CTL line (Table V).

In conclusion, these results clearly show that the H-Y antigen can serve as a target structure on HPC for cytotoxic T cell responses, and may therefore conceivably play a role in bone marrow graft rejection in man. Such a risk could be particularly pertinent for HLA-A2 positive female recipients, since there seems to be a relationship between anti-H-Y responsiveness and the presence of the HLA-A2 antigen (44). In renal transplantation, for example, it was found that female recipients of male donor kidneys had a decreased transplant survival if recipient and donor shared the HLA-A2 antigen (45). Furthermore, in recent years we isolated anti-H-Y CTL lines from five different patients, four of which were found to be restricted by HLA-A2 while only one CTL-line showed a different restricting element, i.e., HLA-A1 (unpublished data). Although these numbers are small they may indicate that HLA-A2 restricted recognition of the H-Y antigen may be more frequent than H-Y antigen recognition in the context of other HLA-antigens. This may implicate that, in particular after T cell depletion of bone marrow grafts, HLA-A2 positive female recipients of a bone marrow graft from an HLA-A2 positive male donor may be at greater risk of rejecting their transplants, especially if they have been extensively transfused or gave birth to male children.

Acknowledgments

This study was supported in part by grants from the J. A. Cohen Institute for Radiopathology and Radiation Protection, and the Dutch Foundation for Medical Research and Health (Medigon).

References

1. Gale, R. P., R. E. Champlin, S. A. Feig, and J. H. Fitchen. 1981. Aplastic anemia: biology and treatment. *Ann. Intern. Med.* 95:477–494.

2. Storb, R., E. D. Thomas, C. D. Buckner, F. R. Appelbaum, R. A. Clift, H. J. Deeg, K. Doney, J. A. Hansen, R. L. Prentice, J. E. Sanders, P. Stewart, K. M. Sullivan, and R. P. Witherspoon. 1984. Marrow transplantation for aplastic anemia. *Semin. Hematol.* 21:27–35.

3. Thomas, E. D., C. D. Buckner, R. A. Clift, A. Fefer, F. L. Johnson, P. E. Neiman, G. E. Sale, J. E. Sanders, J. W. Singer, H. Shulman, R. Storb, and P. L. Weiden. 1979. Marrow transplantation for acute nonlymphoblastic leukemia in first remission. *N. Engl. J. Med.* 301:597–599.

4. Thomas, E. D., J. E. Sanders, N. Flournoy, F. L. Johnson, C. D. Buckner, R. A. Clift, A. Fefer, B. W. Goodell, R. Storb, and P. L. Weiden. 1979. Marrow transplantation for patients with acute lymphoblastic leukemia in remission. *Blood.* 54:468–476.

5. Glucksberg, H., R. Storb, A. Fefer, C. D. Buckner, P. E. Neiman, R. A. Clift, K. G. Lerner, and E. D. Thomas. 1974. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation.* 18:295–304.

6. Thomas, E. D., R. Storb, R. A. Clift, A. Fefer, F. L. Johnson, P. E. Neiman, K. G. Lerner, H. Glucksberg, and C. D. Buckner. Bone marrow transplantation. *N. Engl. J. Med.* 292:832–843.

7. Champlin, R. E., S. A. Feig, and R. P. Gale. 1984. Case problems in bone marrow transplantation. I. Graft failure in aplastic anemia: its biology and treatment. *Exp. Hematol.* 12:728–733.

8. Storb, R., R. L. Prentice, E. D. Thomas, F. R. Appelbaum, H. J. Deeg, K. Doney, A. Fefer, B. W. Goodell, E. Mickelson, P. Stewart, K. M. Sullivan, and R. P. Witherspoon. 1983. Factors associated with graft rejection after HLA-identical marrow transplantation for aplastic anaemia. *Br. J. Haematol.* 55:573–585.

9. Storb, R., P. L. Weiden, M. L. Schroeder, T. C. Graham, K. G. Lerner, and E. D. Thomas. 1976. Marrow grafts between canine littermates, homozygous or heterozygous for lymphocyte defined histocompatibility antigens. *Transplantation.* 21:299–306.

10. Hows, J. M., J. Yin, L. Jones, J. Apperley, K. Econimou, D. C. O. James, J. Batchelor, J. Goldman, and E. C. Gordon-Smith. 1986. Allogeneic bone marrow transplantation with volunteer unrelated donors. *Bone Marrow Transplant.* 1(Suppl. 1):125. (Abstr.)

11. Fitchen, J. H., K. A. Foon, and M. J. Cline. 1981. The antigenic characteristics of hematopoietic stem cells. *N. Engl. J. Med.* 305:17–25.

12. Falkenburg, J. H. F., J. Jansen, N. Van der Vaart-Duinkerken, W. F. J. Veenhof, J. Blotkamp, H. M. Goselink, J. Parlevliet, and J. J. van Rood. 1984. Polymorphic and monomorphic HLA-DR determinants on human hematopoietic progenitor cells. *Blood.* 63:1125–1132.

13. Falkenburg, J. H. F., W. E. Fibbe, H. M. Goselink, J. J. van Rood, and J. Jansen. 1985. Human hematopoietic progenitor cells in long-term cultures express HLA-DR antigens, and lack HLA-DQ antigens. *J. Exp. Med.* 162:1359–1369.

14. Martin, P. J., J. A. Hansen, C. D. Buckner, J. E. Sanders, H. J. Deeg, P. Stewart, F. R. Appelbaum, R. A. Clift, A. Fefer, R. P. Witherspoon, M. S. Kennedy, K. M. Sullivan, N. Flournoy, R. Storb, and E. D. Thomas. 1985. Effects of in vitro depletion of T cells in HLA-identical allogeneic marrow grafts. *Blood.* 66:664–672.

15. Hows, J., J. Apperley, J. Yin, G. Hale, H. Waldman, J. Goldman, and E. Gordon-Smith. 1985. T-cell depletion with campath 1 to prevent GVHD. *Exp. Hematol.* 13(Suppl. 17):114.

16. Barrett, A. J., C. H. Poynton, P. Flanagan, P. J. Shaw, and D. McCarthy. 1986. Factors associated with rejection in bone marrow depleted of T-lymphocytes by Campath-1 monoclonal antibody. *Bone Marrow Transplantation.* 1(Suppl. 1):95 (Abstr.).

17. Patterson, J., H. G. Prentice, M. Gilmore, H. Blacklock, M. K. Brenner, G. Janossy, D. Skeggs, K. Ivory, A. V. Hoffbrand, J. Apperley, J. Goldman, A. Burnett, J. Gribben, M. Alcorn, C. Pearson, I. McVickers, I. Hann, C. Reid, D. Wardle, A. Bacigalupo, and A. G. Robertson. 1985. Analysis of rejection in HLA-matched T-depleted bone marrow transplants. *Exp. Hematol.* 13(Suppl. 17):117.

18. Sondel, P. M., M. J. Bozdech, M. E. Trigg, R. Hong, J. L. Finlay, P. C. Kohler, W. Longo, J. A. Hank, R. Billing, R. Steeves, and B. Flynn. 1985. Additional immunosuppression allows engraftment following HLA-mismatched T cell-depleted bone marrow transplantation for leukemia. *Transplant Proc.* 17:460–461.

19. O'Reilly, R. J., B. Shank, N. Collins, N. Kernan, J. Brochstein, C. Keever, R. Dinsmore, D. Kirkpatrick, H. Castro-Malaspina, J. Cunningham, N. Flomenberg, and R. Burns. 1985. Increased total body irradiation (TBI) abrogates resistance to HLA-matched marrow grafts depleted of T cells by lectin agglutination and E-rosette depletion (SBA-E-BMT). *Exp. Hematol.* 13:406. (Abstr.)

20. Or, R., Z. Weshler, G. Lugassy, D. Steiner-Salz, E. Galun, L. Weiss, S. Samuel, A. Poliack, E. A. Rachmilewitz, H. Waldmann, and S. Slavin. 1985. Total lymphoid irradiation (TLI) as adjunct immunosuppressor for preventing late graft failure (LGF) associated with T-cell depleted marrow allograft. *Exp. Hematol.* 13:409. (Abstr.)

21. Reisner, Y., I. Ben-Bassat, D. Douer, A. Kaploon, E. Schwartz, and B. Ramot. 1985. A primate pre-clinical model for bone marrow transplantation: definite proof for host radioresistant clonable T cells. *Exp. Hematol.* 13:321 (Abstr.).

22. Butturini, A., R. C. Seeger, and R. P. Gale. 1986. Recipient immune-competent T-lymphocytes can survice intensive conditioning for bone marrow transplantation. *Blood.* 68:954–956.

23. Simpson, E., P. Chandler, E. Goulmy, C. M. Disteche, M. A. Ferguson-Smith, and D. C. Page. 1987. Separation of the genetic loci for the H-Y antigen and for testis determination on human Y chromosome. *Nature (Lond.).* 326:876–878.

24. Eichwald, E. J., and C. R. Slimser. 1955. *Transplant. Bull.* 2:148–249.

25. Goulmy, E., A. Termijtelen, B. A. Bradley, and J. J. van Rood. 1977. Y-antigen killing by T-cells of women is restricted by HLA. *Nature (Lond.).* 266:544–545.

26. Simpson, E. 1983. The role of H-Y as a minor transplantation antigen. *In* T-lymphocytes Today. J. R. Inglis, editor. Elsevier, Amsterdam. pp. 135–144.

27. Goulmy, E., E. Blokland, J. J. van Rood, D. Charmot, B. Malissen, and C. Mawas. 1980. Production, expansion, and clonal analysis of T cells with specific HLA-restricted male lysis. *J. Exp. Med.* 152:182s–190s.

28. Goulmy, E. 1982. HLA-A, -B restriction of cytotoxic T cells. *In* HLA typing: methodology and clinical aspects. Vol. 2, S. Ferrone and B. G. Solheim, editors. CRC Press, New York. 105–122.

29. Mathieson, B. J., S. O. Sharrow, P. S. Campbell, R. Asofsky. 1979. A Lyt differentiated thymocyte subpopulation detected by flow microfluorometry. *Nature (Lond.).* 277:478–480.

30. Falkenburg, J. H. F., W. E. Fibbe, N. Van der Vaart-Duinkerken, M. E. Nichols, P. Rubinstein, and J. Jansen. 1985. Human erythroid progenitor cells express Rhesus antigens. *Blood.* 66:660–663.

31. Voogt, P. J., W. E. Fibbe, W. F. J. Veenhof, A. Brand, E. Goulmy, J. J. van Rood, and J. H. F. Falkenburg. 1987. Cell-mediated lysis of human hematopoietic progenitor cells. *Leukemia.* 1:427–431.

32. Iscove, N. N., J. S. Senn, J. E. Till, and E. A. McCulloch. 1971. Colony formation by normal and leukemic human marrow cells in culture: effect of conditioned medium from human leucocytes. *Blood.* 37:1–5.

33. Haak, H. L., H. M. Goselink, W. F. J. Veenhof, J. Blotkamp, and J. Jansen. 1984. The in-vitro growth pattern of human bone mar-

row in methylcellulose stimulated by different concentrations of conditioned medium. *Scand. J. Haematol.* 32:515–524.

34. Ash, R. C., R. A. Detrick, and E. D. Zanjani. 1981. Studies of human pluripotential hemopoietic stem cells (CFU-GEMM) in-vitro. *Blood.* 58:309–316.

35. Messner, H. A. 1984. Human stem cells in culture. *Clin. Hematol.* 13:393–404.

36. Madsen, M., and H. E. Johnson. 1979. A methodological study of E-rosette formation using AET-treated sheep red blood cells. *J. Immunol. Methods.* 27:61–74.

37. De Boer, M., R. Reyneke, R. J. van de Griend, J. A. Loos, and D. Roos. 1981. Large-scale purification and cryopreservation of human monocytes. *J. Immunol. Methods.* 43:225–239.

38. Lundgren, G., Ch. F. Zukoshi, and G. Moller. 1968. Differential effects of human granulocytes and lymphocytes on human fibroblasts in vitro. *Clin. Exp. Immunol.* 3:817–836.

39. Storb, R., K. C. Doney, E. D. Thomas, F. R. Appelbaum, C. D. Buckner, R. A. Clift, H. J. Deeg, B. W. Goodel, R. Hackman, J. A. Hansen, J. Sanders, K. Sullivan, P. L. Weiden, and R. P. Witherspoon. 1982. Marrow transplantation with or without donor buffy coat cells for 65 transfused aplastic anemia patients. *Blood.* 59:236–246.

40. Kernan, N. A., C. Bordignon, I. Cunningham, H. Castro-Malaspina, J. Brochstein, B. Shank, N. H. Collins, N. Flomenberg, B. Dupont, and R. J. O'Reilly. 1987. Recipient age and donor sex are factors for graft-failure (GF) following T cell depleted (SBA^-E^-) BMT for leukemia. *Blood.* 70(Suppl. 1):309a. (Abstr.)

41. Cook, K. M. 1974. Distribution of HL-A antigens on blood cells. *Tissue Antigens.* 4:202–209.

42. Sieff, C., D. Bicknell, G. Caine, J. Robinson, G. Lam, and M. F. Greaves. 1982. Changes in cell surface antigen expression during hematopoietic differentiation. *Blood.* 60:703–713.

43. Drew, S. I., B. M. Carter, P. I. Terasaki, F. Naiem, D. S. Nathanson, B. Abromowith, and R. P. Gale. 1978. Cell surface antigens detected on mature and leukemic granulocytic populations by cytotoxicity testing. *Tissue Antigens.* 12:75–86.

44. Goulmy, E. 1985. Class-I-restricted human cytotoxic T-lymphocytes directed against minor transplantation antigens and their possible role in organ transplantation. *Prog. Allergy.* 36:44–72.

45. Goulmy, E., B. A. Bradley, Q. Lansbergen, and J. J. van Rood. 1978. The importance of H-Y incompatibility in human organ transplantation. *Transplantation.* 25:315–319.