Minor Histocompatibility Antigens HA-1–, -2–, and -4–, and HY-Specific Cytotoxic T-Cell Clones Inhibit Human Hematopoietic Progenitor Cell Growth by a Mechanism That Is Dependent on Direct Cell-Cell Contact

By W.A.F. Marijt, W.F.J. Veenhof, E. Goulmy, R. Willemze, J.J. van Rood, and J.H.F. Falkenburg

HLA-identical bone marrow transplantation (BMT) may be complicated by graft-versus-host disease or graft rejection. Both complications are thought to be initiated by recognition of minor histocompatibility (mH) antigens by HLA-restricted mH-antigen–specific T lymphocytes. Using HLA-A2–restricted mH antigens HA-1–, -2–, and -4–, and HY-specific cytotoxic T lymphocyte (CTL) clones, we studied the recognition by these CTL clones of interleukin-2 (IL-2)–stimulated T cells (IL-2 blasts), BM mononuclear cells (BMMNCs), and hematopoietic progenitor cells (HPCs). We showed that, when IL-2 blasts from the BM donors who were investigated were recognized by the HA-1–, -2–, and -4–, and HY-specific CTL clones, their BMMNCs and HPCs were recognized as well by these CTL clones, resulting in antigen-specific growth inhibition of erythrocyte burst-forming units (BFU-E), colony-forming units-granulocyte (CFU-G), and CFU-macrophage (CFU-M). The HA-2–specific CTL clone, however, inhibited BFU-E and CFU-G growth from four donors to a lesser extent than from two other donors. We further investigated whether inhibitory cytokines released into the culture medium by the antigen-specific stimulated CTLs or by stimulated BMMNCs were responsible for suppression of HPC growth or whether this effect was caused by direct cell-cell contact between CTLs and HPCs. HPC growth inhibition was only observed after preincubation of BMMNCs and CTLs together for 4 hours before plating the cells in semisolid HPC culture medium. When no cell-cell contact was permitted before plating, neither antigen-stimulated CTL nor antigen-nonstimulated CTLs provoked HPC growth inhibition. Culturing BMMNCs in the presence of supernatants harvested after incubation of BMMNCs and CTL clones together for 4 or 72 hours did also not result in HPC growth inhibition. Both suppression of HPC growth and lysis of IL-2 blasts and BMMNCs in the ^{51}Cr-release assay appeared to be dependent on direct cell-cell contact between target cells and CTLs and were not caused by the release of inhibitory cytokines into the culture medium by antigen-specific stimulated CTLs or by stimulated BMMNCs. Our results show that mH-antigen–specific CTLs can inhibit HPC growth by a direct cytolytic effect and may therefore be responsible for BM graft rejection after HLA-identical BMT.

HLA-IDENTICAL bone marrow transplantation (BMT) is frequently complicated by acute graft-versus-host disease (GVHD). To decrease the incidence and severity of acute GVHD, BM grafts are depleted of mature T cells.[1,2] However, T-cell depletion is associated with an increased incidence of graft rejection and graft failure.[3-6] Immunologically mediated graft rejection is thought to be caused by residual immunocompetent recipient T cells that recognize allo-antigens expressed on donor hematopoietic progenitor cells (HPCs). After haploidentical or partially matched unrelated BMT, these T cells may recognize HLA class I or II antigens.[7-9] After HLA genotypically identical BMT or matched unrelated BMT, residual host T cells may react with minor histocompatibility (mH) antigens expressed on donor cells.[10] mH antigens are presented in the context of HLA antigens to HLA class I or II restricted mH-antigen–specific T cells.[11,12] It is important to know whether mH antigens expressed on human HPCs can be recognized by mH-antigen–specific cytotoxic T lymphocytes (CTLs) and by what mechanism such CTLs suppress HPC growth. It might then be possible to anticipate or prevent the occurrence of graft rejection after HLA-identical BMT by residual recipient antidonor CTL.

Previously, we have shown the recognition of human HPCs by HA-3 and HY-specific CTL lines.[13,14] Expression of HA-1, -2, -4, and -5 could not be clearly shown, although at high effector:target (E:T) ratios and after prolonged incubation times partial inhibition of HPC growth was observed. However, it has been shown that increased E:T ratios result in increased antigen-nonspecific inhibition of HPC growth.[15] Based on these results, we concluded that mH antigens may be differentially expressed on human HPCs. Recently, we showed the recognition of clonogenic leukemic precursor cells by HA-1–, -2–, -4–, and -5–specific CTL clones.[16] Furthermore, we have reported the recognition of HPCs by an mH-antigen–specific CTL line.[17] This CTL line appeared to recognize a large panel of unrelated HLA-A2–positive individuals in a pattern analogous to the distribution of the mH antigen HA-2 expression in the population.[18] We therefore hypothesized that the lack of recognition by the HA-2–specific CTL line in the previous study[14] may have been caused by relatively low frequencies of the respective mH-antigen–specific CTLs to ensure a sufficient E:T ratio to inhibit HPC growth. The recent cloning of the mH antigen HA-1–, -2–, -4–, and -5–, and HY-specific CTL lines[18] provided us with sensitive cellular reagents to reinvestigate the expression of mH antigens on HPCs in more detail.

Conflicting reports have been published about the mecha-

From the Laboratory of Experimental Hematology, Department of Hematology, and the Department of Immunohematology and Bloodbank, University Medical Center, Leiden, The Netherlands.

Submitted June 1, 1993; accepted August 19, 1993.

Supported in part by grants from the J.A. Cohen Institute for Radiopathology and Radiation Protection. J.H.F.F. is a special fellow of the Royal Netherlands Academy of Arts and Sciences.

Address reprint requests to W.A.F. Marijt, MD, Department of Hematology, Building 1, C2-R, University Medical Center, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

0006-4971/93/8212-0011$3.00/0

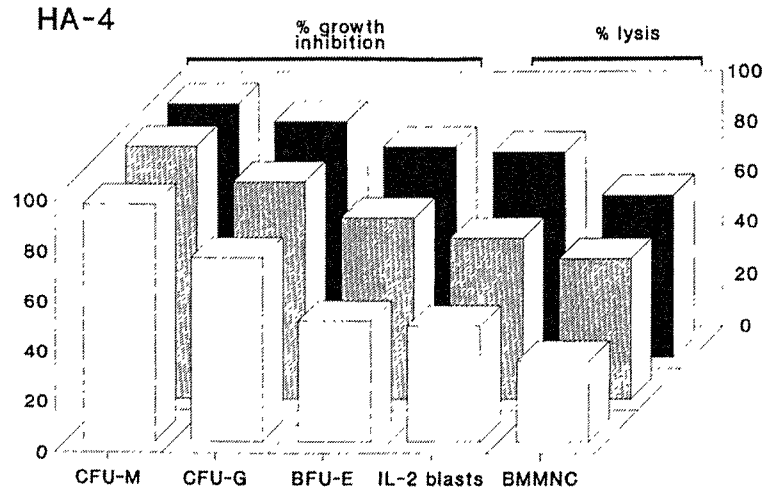

Fig 1. Recognition of different target cells from one donor by the HA-4–specific CTL clone using three E:T ratios ([□] 0.3:1, [▨] 1:1, [■] 3:1). BFU-E, CFU-G, and CFU-M were tested in the HPC growth inhibition assay, and IL-2 blasts and BMMNCs in the ^{51}Cr-release assay.

nism of HPC growth suppression by effector cell populations. For instance, CTLs may produce a variety of cytokines on antigen-specific stimulation by target cells.[19-22] Alternatively, CTLs may stimulate target BM mononuclear cells (BMMNCs) to produce cytokines. Thus, incubation of CTLs with BM target cells may result in HPC growth inhibition caused by a suppressive effect of cytokines, as reported by other investigators.[21,23] On the other hand, cell-cell contact between CTLs and antigen-positive target cells leads to CTL stimulation, resulting in direct target cell death.[24-26] We and others have illustrated that cell-cell contact is important to obtain HPC growth inhibition, suggesting that the suppression may be caused by target cell lysis.[15,27] However, Bunjes et al[28] showed that both recipient peripheral blood lymphocytes (PBLs) collected after BM graft rejection as well as supernatants harvested after incubation of recipient PBLs in medium alone or in the presence of concanavalin A (con A) suppressed HPC growth.

In this study, we describe, using the HLA-A2–restricted mH antigen HA-1–, -2–, and -4–, and HY-specific CTL clones, the recognition of these mH antigens on interleukin-2 (IL-2) blasts, BM cells, and HPCs. We show that antigen-specific growth inhibition of HPCs requires direct cell-cell contact between HPCs and CTLs and is not caused by secretion of HPC growth-inhibitory cytokines by antigen-specifically stimulated CTLs or activated target cells.

MATERIALS AND METHODS

Collection of cells. Normal human BM was obtained, after informed consent, from donors for BMT by aspiration from the posterior iliac crests. BMMNCs and PBLs were isolated and cryopreserved as described.[17] PBLs were thawed immediately before use and resuspended in RPMI plus 15% pooled human serum (RPMI plus serum). BMMNCs were thawed, washed, and cultured for 18 hours in RPMI plus 15% human serum before use.

HLA types of BM donors The HLA types of the BM donors were donor 1: A1/2, B8/35, C4/7, DR3/8, DQ2; donor 2: A1/2, B8/15, C3/7, DR3/4, DQ2/3; donor 3: A1/2, B17/35, C4/6, DR7/8, DQ3; donor 4: A1/2, B8/40, C2/7, DR3/4, DQ2/3; donor 5: A2, B15/40, C2/3, DR5/7/11, DQ2/3/7; and donor 6: A2/28, B37/53, C4/6, DR9/13, DQ1/3.

^{51}Cr-release assay. Target cells were either BMMNCs or recombinant IL-2 (rIL-2)–stimulated T cells (IL-2 blasts) that were generated by culturing 10^7 PBLs in 10 mL RPMI plus 15% serum and 0.2% phytohemagglutinin (PHA; Difco Laboratories, Detroit, MI) for 3 days. The cells were then washed and further cultured in the absence of PHA in medium consisting of RPMI plus 15% serum and 300 IU rIL-2/ml (T-cell culture medium). After 2 to 3 days of culture, the cells were used as targets in a ^{51}Cr-release assay.

HA-1–, -2–, and -4–, and HY-specific CTL clones, generated as described,[18] and HLA-A1– and -A2–specific CTL clones were cultured in T-cell culture medium. They were used as effector cells 7 days after stimulation with irradiated (50 Gy) Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (EBV-LCLs) derived from the original stimulator cells and allogeneic PBLs. Eighteen hours before use, fresh T-cell culture medium was added to the CTL clones in the absence of feeder cells. Standard ^{51}Cr-release assays were performed as described[29] at E:T ratios of 2:1, 1:1, and 0.3:1.

HPC growth inhibition assay. BMMNCs were depleted of T cells by 2-aminoethylisothiouroniumbromide–pretreated sheep red blood cells as described.[30] A quantity of 1.25×10^5 BMMNCs in 0.1 mL HPC culture medium consisting of IMDM supplemented with 30 vol% blood group AB heparin plasma and 0.5% bovine serum albumin (BSA), 0.47 g/L transferrin, and 5×10^{-5} mol/L mercaptoethanol was mixed with 0.1 mL of HPC culture medium containing CTLs at E:T ratios of 2:1, 1:1, and 0.3:1. CTLs were irradiated (20 Gy) before use to prevent colony formation by these cells. The cell mixture was centrifuged (1,000g for 15 seconds) to establish direct cell-cell contact between CTLs and BMMNCs and then incubated for 4 hours in a fully humidified atmosphere of 5% CO_2 at 37°C. After incubation, the cells were resuspended at a final volume of 1.4 mL of HPC culture medium supplemented with glycosilated recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF; Sandoz Ltd, Basel, Switzerland) at a final concentration of 10 ng/mL, rIL-3 (Sandoz) at a final concentration of 50 ng/mL, 2 IU of recombinant erythropoietin (Cilag AG International, Zug, Switzerland), and methylcellulose at a final concentration of 1.3%. Subsequently, 1.15 mL of this suspension was plated in 30-mm plastic dishes, incubated in a fully humidified atmosphere of 5% CO_2 and 37°C, and cultured for 18 days. The number of erythrocyte burst-forming units (BFU-E), colony-forming units-granulocyte (CFU-G), and CFU-macrophage (CFU-M), defined as cell aggregates of more than 20 cells, were scored on day 18 under an inverted microscope. To establish the necessity for direct cell-cell

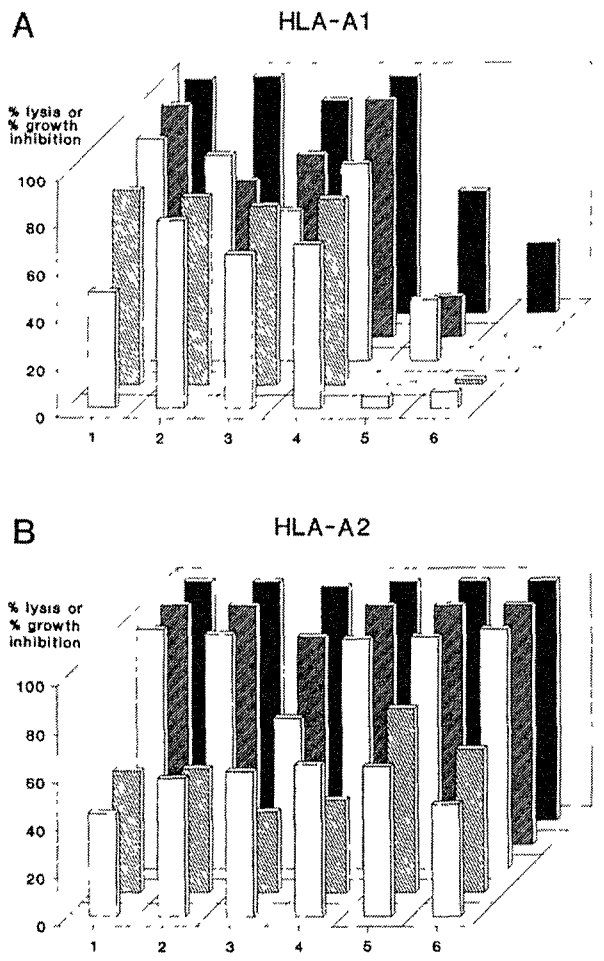

Fig 2. Recognition by the HLA-A1– and -A2–specific CTL clones of (□) BMMNCs and (▨) IL-2 blasts (tested in the ^{51}Cr-release assay and expressed as a percentage of lysis) and on (□) BFU-E, (▨) CFU-G, and (■) CFU-M (tested in the HPC growth inhibition assay and expressed as a percentage of growth inhibition relative to HPC growth of untreated control BMMNCs). E:T ratio shown is 2:1.

contact between CTLs and BMMNCs, and to exclude the possibility of antigen-nonspecific inhibition of HPC growth caused by the presence of CTLs in the semisolid culture medium, CTLs and BMMNCs were separately preincubated for 4 hours and mixed immediately before plating.

To analyze whether soluble factors, secreted during the incubation period, were responsible for antigen-specific HPC growth inhibition, three different protocols were used. First, 5×10^4 irradiated BMMNCs and 1×10^5 irradiated CTLs were incubated for either 4 or 72 hours in HPC culture medium in a fully humidified atmosphere consisting of 5% CO_2 at 37°C. Subsequently, supernatants of this cell mixture were added to 5×10^4 BMMNCs from the same BM donor and, after 18 days of culture, BFU-E, CFU-G, and CFU-M colonies were scored. Second, irradiated BMMNCs were added to CTLs and incubated for 4 hours. Subsequently, these antigen-specific stimulated CTLs were added to nonirradiated BMMNCs from the same donor immediately before plating and, after 18 days of culture, BFU-E, CFU-G, and CFU-M colonies were scored. To control for cell crowding effects, BMMNCs and CTLs were incubated for 4 hours, irradiated BMMNCs from the same donor were added, the cell mixture was plated, and, after 18 days of culture, BFU-E, CFU-G, and CFU-M colonies were scored. Third, to exclude the possibility of HPC growth inhibition caused by labile factors, irradiated antigen-positive BMMNCs, irradiated antigen-specific CTLs, and nonirradiated antigen-negative BMMNCs were incubated for 4 hours, plated, and, after 18 days of culture, BFU-E, CFU-G, and CFU-M colonies were scored. Alternatively, irradiated antigen-specific CTLs and antigen-negative BMMNCs were incubated for 4 hours, plated, and, after 18 days of culture, BFU-E, CFU-G, and CFU-M colonies were scored. As a control for antigen-specific HPC growth inhibition, nonirradiated antigen-positive BMMNCs and irradiated antigen-specific CTLs were incubated for 4 hours, plated, and, after 18 days of culture, BFU-E, CFU-G, and CFU-M colonies were scored.

RESULTS

mH antigens HA-1, -2, and -4, and HY are expressed on HPC. In Fig 1, a representative example of the dose-dependent lysis of BMMNCs and IL-2 blasts and growth inhibition of HPCs by the mH antigen HA-4–specific CTL clone is shown. CFU-M and CFU-G growth inhibition was still maximal at low E:T ratios, illustrating the high sensitivity of this assay. In each experiment, IL-2 blasts, BMMNCs, and HPCs from a single BM donor were used as target cells, and the HLA-A1– and -A2–, HA-1–, -2–, and -4–, and HY-specific CTL clones as effector cells in E:T ratios of 2:1, 1:1, and 0.3:1. Results obtained with an E:T ratio of 2:1 are displayed in Figs 2, 3, and 4. BMMNCs, IL-2 blasts, BFU-E, CFU-G, and CFU-M from all six BM donors tested were recognized by the HLA-A2– (Fig 2B), and mH antigen HA-2–specific CTL clones (Fig 3B). All target cells from donors 1, 2, 3, and 4 were recognized by the HLA-A1–specific CTL clone (Fig 2A). The mH antigen HA-1–specific CTL clone recognized all target cells from donors 1, 2, 3, and 6 (Fig 3A). All target cells from donors 4, 5, and 6 were recognized by the mH antigen HA-4– and HY-specific CTL clones, the latter also recognizing all target cells from donor 2 (Fig 3C and D). Target cells from donor 1 could not be tested by these two CTL clones. The recognition of IL-2 blasts by the HLA-A1– and -A2–, mH antigens HA-1– and -4–, and HY-specific CTL clones correlated with the recognition of BMMNCs, BFU-E, CFU-G, and CFU-M by these CTL clones. The highly lytic HLA-A1– and HA-1–specific CTL clones showed limited antigen-nonspecific HPC growth inhibition in some HLA-A1– or HA-1–negative donors (donors 5 and 6 and donors 4 and 5, respectively), whereas no significant lysis of their IL-2 blasts and BMMNCs was detected. Recognition of target cells from the HLA-A1–positive donors 1 through 4 by the HLA-A1–specific CTL clone (Fig 2A) and from the HLA-A2–positive donors 1 through 6 by the HLA-A2–specific CTL clone (Fig 2B) provided the positive controls for the assays. The negative controls were formed by effector-target cell combinations of which the target cell lacked either the HLA restriction element or the mH antigen, eg, donors 5 and 6 in Fig 2A, donors 2 and 3 in Fig 3C, and the female donor 3 in Fig 3D.

The HA-2–specific CTL clone recognized IL-2 blasts, BMMNCs, BFU-E, CFU-G, and CFU-M from all donors (Fig 3B). However, BFU-E and CFU-G from donors 3

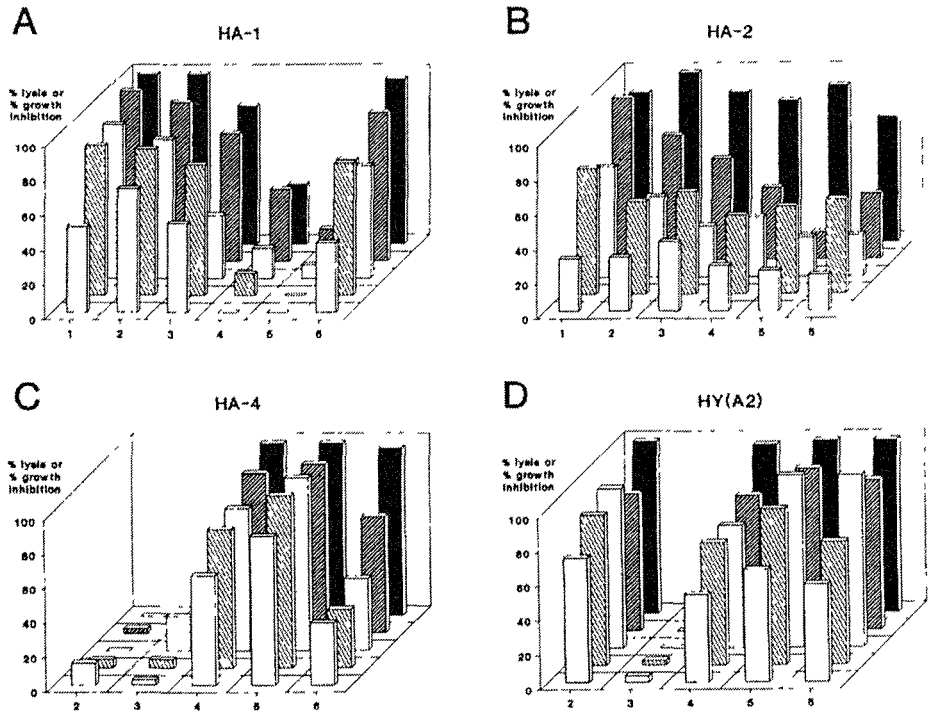

Fig 3. Recognition by the HA-1–, -2–, and -4–, and HY-specific CTL clones of (□) BMMNC, (▨) IL-2 blasts, (▯) BFU-E, (▨) CFU-G, and (■) CFU-M. For details see legend to Fig 2.

through 6 were recognized to a lesser extent. The overall percentage lysis of BMMNCs from the donors by the HA-2–specific CTL clone was also lower than the percentage lysis of BMMNCs by the other CTL clones. HPCs from donors 3 through 6 were clearly recognized by other CTL clones whenever they expressed the appropriate antigen. Impaired expression of the HLA-A2 restriction molecule on HPCs of the HA-2–positive individuals appeared not to be an explanation for the diminished or absent recognition, as shown by the high percentage of growth inhibition of HPCs from donors 3 through 6 by the HLA-A2–specific CTL clone (Fig 2B).

HPC growth inhibition depends on direct cell-cell contact Table 1 and Figs 4 and 5 show the results of the experiments performed to investigate whether direct cell-cell contact was necessary to inhibit HPC growth or whether cytokines produced by antigen-specifically stimulated CTL or activated target cells were responsible for suppression of HPC growth. Supernatants harvested after 4 hours (Fig 4A and B; row IV) or 72 hours (Table 1) of incubation of effector and target cells did not inhibit HPC growth. Preincubating CTLs and BMMNCs separately before plating, thus avoiding antigen-specific stimulation, inhibited HPC growth only to a limited extent (Fig 4A and B; row I). CTL that were antigen-specifically stimulated by preincubation with irradiated BMMNCs and were mixed immediately before plating with nonirradiated BMMNCs did not inhibit HPC growth either (Fig 4A and B; row III). However, only after CTLs and BMMNCs were preincubated for 4 hours was significant antigen-specific HPC growth inhibition observed (Fig 4A and B; row II); the addition of irradiated BMMNCs to control for cell-crowding effects did not influence HPC growth inhibition. Figure 5 shows that incubation for 4 hours of the HLA-A2–restricted HA-2–specific CTLs with (Fig 5, row I) or without (Fig 5, row II) HA-2–positive irradiated BMMNCs and HLA-A2–negative nonirradiated BMMNCs did not induce HPC growth inhibition. Incubation of nonirradiated HA-2–positive BMMNCs with irradiated HA-2–specific CTLs for 4 hours resulted in almost complete HPC growth inhibition (Fig 5, row III). These results illustrate that HPC growth inhibition by HLA class I and mH-antigen-specific CTL clones is not caused by soluble factors released into the culture medium but that direct cell-cell contact between HPCs and CTLs is required.

DISCUSSION

In this report, we show the recognition of mH antigens on IL-2 blasts, BMMNCs, and HPCs by the HLA-A2–restricted mH antigen HA-1–, -2–, and -4–, and HY-specific CTL clones. Previously, we reported the recognition of human HPCs by CTL lines specific for the HLA-A1–restricted mH antigen HA-3 and the HLA-A1–, A2–, or B7-restricted mH antigen HY, but not by the HA-1–, -2–, -4–, and -5–specific CTL lines.[13,14] In that report, recognition of HPCs by HA-1–, -2–, -4–, and -5–specific CTL lines could only be detected to a limited extent using high E:T ratios, resulting in significant antigen-nonspecific HPC growth inhibition. This antigen-nonspecific suppression of HPC growth may have been caused by high concentrations of cytokines produced by the large number of CTLs present in the cultures, thus inducing decreased sensitivity and specificity of the cellular assay. All HPC growth inhibition assays in these studies were performed using CTL lines. CTL lines may consist of multiple clones with different specificities result-

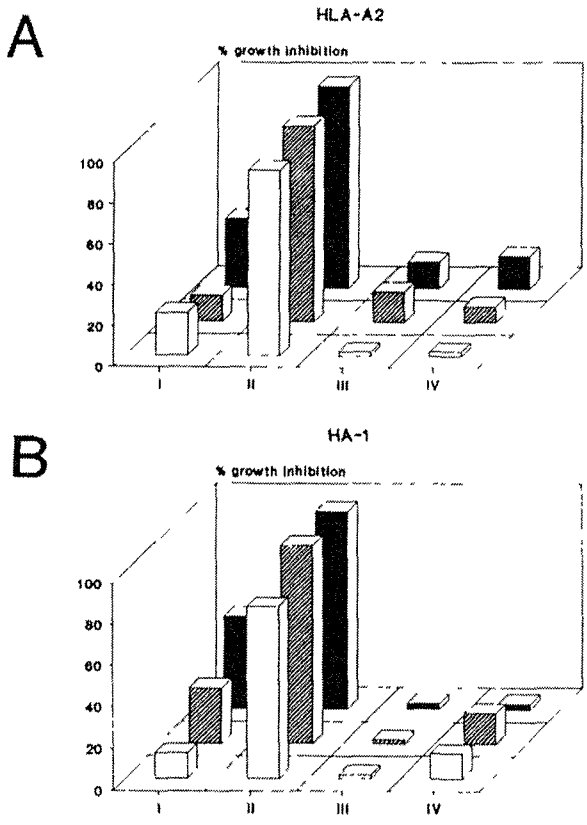

Fig 4. Direct cell-cell contact-dependent HPC growth inhibition by HLA-A2- (A) and HA-1-specific (B) CTL clones. (I) BMMNCs and CTLs were preincubated separately and mixed immediately before plating. (II) BMMNCs and CTLs were first preincubated together for 4 hours and then irradiated BMMNCs were added as a control for cell-crowding effects. Thereafter, the cell suspensions were cultured for HPC growth. (III) Irradiated BMMNCs were incubated with CTLs for 4 hours and then nonirradiated BMMNCs were added and the cell suspensions were cultured for HPC growth. (IV) CTLs were incubated with BMMNCs in HPC culture medium for 4 hours, whereafter the supernatants were harvested, added to BMMNCs from the same donor, and cultured for HPC growth. Bars represent the mean of two experiments. E:T ratio is 2:1. (□) BFU-E; (▨) CFU-G; (■) CFU-M.

ing in a "dilution" of the relevant CTL clone and, consequently, in a lower E:T ratio. This "dilution" results apparently not in decreased lysis or recognition when the target cell suspension is relatively homogeneous, as is the case with IL-2 blasts. However, HPCs as target cells do not only occur at a low frequency of 1% in BM cell suspensions (500 colonies formed by 5×10^4 plated BMMNCs) but are also mixed with 99% BM cells expressing the relevant antigen and thus functioning as "cold targets." The HA-3- and the HY-specific CTL lines studied previously were highly cytotoxic, and this may have compensated for the low E:T ratios. Using the HA-1-, -2-, and -4-, and HY-specific CTL clones[18] instead of CTL lines, the sensitivity of the assay appeared to be significantly improved. Recognition of IL-2 blasts and BMMNCs from the donors tested in the ^{51}Cr-release assay by the mH-antigen-specific CTL clones was associated with growth inhibition of HPCs from the same donor. In some instances, the IL-2 blasts as well as the BMMNCs and HPCs were not recognized by the mH-antigen-specific CTL clones, suggesting that these donors lacked expression of the mH antigen on their lympho-hematopoietic cells. However, the HA-2-specific CTL clone recognized BFU-E and CFU-G from donors 3 through 6 to a lesser extent. Lysis of BMMNCs from all donors by the HA-2-specific CTLs was lower as well. This was not caused by diminished sensitivity to lysis of BMMNCs, BFU-E, and CFU-G from these donors, as these targets were clearly recognized by other CTL clones, eg, HA-4- and HY-specific CTLs. Decreased expression of the HLA-A2 restriction element was therefore also not an explanation for this lack of HPC growth inhibition, as was further shown by the effective inhibition of HPC growth of the same donors by the anti-HLA-A2 CTLs. Van der Harst et al[31] observed that the HA-2-specific CTL clone recognized lymphocytic leukemia cells less well. They found that these target cells exhibited low surface expression of the adhesion molecules CD11a/CD18 compared with target cells that were well recognized. Low surface expression of adhesion molecules was probably not an explanation for the low percentage of growth inhibition of BFU-E and CFU-G in our study because each donor was analyzed with all mH-antigen-specific CTL clones. The other mH-antigen-specific CTL clones were capable of inducing strong growth inhibition of BFU-E and CFU-G from donors 3 through 6. Alternatively, the HA-2-specific CTL clone may be more sensitive to impaired adhesion molecule interaction. Decreased recognition of BFU-E and CFU-G from these donors by the HA-2-specific CTL clone may have been caused by a number of other factors either acting alone or in combination. First, it might be related to differentiation and/or maturation of HPCs, resulting in either a loss of HA-2 expression on cells of erythroid and granulocytic lineage or an increase of expression on lymphocytes and macrophages. Differential expression of mH antigens on cells from various tissues has recently been shown by de Bueger et al.[32] HA-1 and -2 could only be detected on cells of lymphocytic origin and monocytes and not on keratinocytes, melanocytes, dermal fibroblasts, kidney epithelial cells, and endothelial cells. Second, expression of HA-2 may be lower on different cell types because of competition with other cell-type-specific peptides for binding to HLA mole-

Table 1 Absence of HPC Growth Inhibition After Incubation of BMMNCs With Supernatant Harvested After Incubation of mH-Antigen-Specific CTLs With mH-Antigen-Positive BMMNCs

CTLs Specific for	BMMNCs Positive for	% HPC Colony Growth		
		BFU-E	CFU-G	CFU-M
HA-1	HA-1	89	111	175
HA-2	HA-2	99	88	132
HA-4	HA-4	88	86	102

Values are supernatant induced HPC growth as a percentage of control HPC growth. Control HPC growth was obtained by culturing of BMMNCs in the presence of IL-3, GM-CSF, and erythropoietin. Results represent the mean of two experiments.

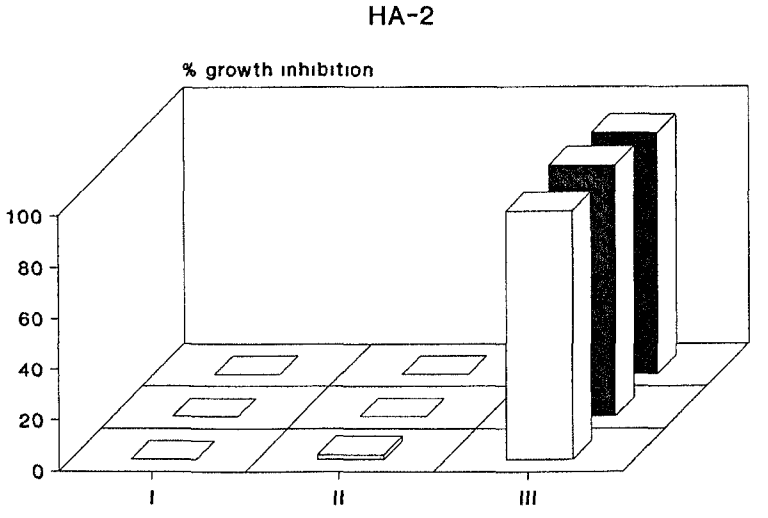

Fig 5 Direct cell-cell contact-dependent HPC growth inhibition by HA-2-specific CTL clone Irradiated HA-2-positive BMMNCs, HLA-A2-restricted HA-2-specific CTLs, and nonirradiated HLA-A2-negative BMMNCs were incubated for 4 hours, plated, and cultured for HPC growth (row I) Alternatively, HA-2-specific CTLs and nonirradiated HLA-A2-negative BMMNCs were incubated for 4 hours, plated, and cultured for HPC growth (row II) As a control for antigen-specific HPC growth inhibition, nonirradiated antigen-positive BMMNCs and antigen-specific CTLs were incubated for 4 hours, plated, and cultured for HPC growth (row III) Bars represent the results of one experiment at an E T ratio of 3 1. (□) BFU-E, (▨) CFU-G, (■) CFU-M

cules Third, expression of HA-2 may depend on expression of another gene product, comparable with expression of the Lewis blood group antigens on erythrocytes being dependent on the secretor genes [33]

It has been postulated that HPC growth inhibition by antigen-specific effector cells is mediated by cytokines secreted into the culture medium and not by target cell lysis after cell-cell contact between effector and target cells Antigen-specific CTLs produce interferon-γ and tumor necrosis factor-β, factors that are inhibitory for HPC growth [34-36] However, they may also produce GM-CSF and IL-2, -3, -4, -5, and -6, of which GM-CSF, IL-3, and IL-5 are potent stimulators of HPC growth [37-40] IL-4 enhances G-CSF-induced CFU-G growth, but is inhibitory for CFU-M growth [41] In the present study, supernatants containing cytokines presumably produced by antigen-specific stimulated CTLs that were harvested after incubation periods of 4 or 72 hours were shown not to be inhibitory for HPC growth Additionally, antigen-specific stimulated CTLs did not inhibit HPC growth when direct cell-cell contact was omitted by preincubating CTLs and BMMNCs separately before mixing and plating the cell suspensions Even stimulation of CTLs by irradiated BMMNCs or stimulation of BMMNCs by CTLs and the likely subsequent production or alteration of production of cytokines induced no HPC growth inhibition both of antigen-positive BMMNCs and of antigen-negative BMMNCs Recognition of target cells by CTLs after establishing direct cell-cell contact will result in direct target cell death [24-26] The inhibitory effect on HPC growth by the CTLs appeared to have taken place during the 4-hour incubation period, and is thus likely to be similar to the lytic effect of the CTL clones on the ^{51}Cr-labeled mature BMMNCs In contrast to the ^{51}Cr-release assay, the HPC growth inhibition assay measures functional capacity of HPCs, present at low frequency in BMMNCs, and may be a better reflection of the in vivo situation

BM graft rejection after HLA-identical BMT is thought to be caused by mH-antigen-specific CTLs These CTLs have been isolated from recipient PBLs in some cases and were inhibitory for donor HPC growth in vitro after establishing cell-cell contact (Marijt et al, manuscript submitted) [10 28 42] Because of recognition of HPCs by all mH-antigen-specific CTLs tested so far and the high probability of mH antigen disparity between HLA-identical siblings,[43 44] many BM graft recipients are likely to be at high risk for BM graft rejection Eradication of residual recipient antidonor CTLs by the conditioning regimen and possibly by the posttransplant immunosuppression is essential for engraftment, except perhaps in homozygotic twin transplantation.

In conclusion, we have shown the expression of the mH antigens HA-1, -2, and -4, and HY on IL-2 blasts, BMMNCs, and HPCs We showed that, for HPC growth inhibition, direct cell-cell contact between HPCs and CTLs during the preincubation period is required, and that this suppression is not caused by secreted cytokines BM graft rejection after HLA-identical BMT may therefore be caused by a direct cytotoxic effect of recipient CTLs that recognize mH antigens expressed on donor HPCs

REFERENCES

1 Hale G, Cobbold S, Waldmann H T cell depletion with Campath-1 in allogeneic bone marrow transplantation Transplantation 45 753, 1988

2 Mitsuyasu RT, Champlin RE, Gale RP, Ho WG, Lenarsky C, Winston D, Selch M, Elashoff R, Giorgi JV, Wells J, Terasaki P, Billing R, Feig S Treatment of donor bone marrow with monoclonal anti-T cell antibody and complement for the prevention of graft-versus-host disease Ann Intern Med 105 20, 1986

3 Kernan NA, Bordignon C, Heller G, Cunningham I, Castro-Malaspina H, Shank B, Flomenberg N, Burns J, Yang SY, Black P, Collins NH, O'Reilly RJ Graft failure after T cell depleted human leukocyte antigen identical marrow transplants for leukemia I Analysis of risk factors and results of secondary transplants Blood 74 2227, 1989

4 Patterson J, Prentice HG, Brenner MK, Gilmore M, Janossy G, Ivory K, Skeggs D, Morgan H, Lord J, Blacklock HA, Hoffbrand AV, Apperley JF, Goldman JM, Burnett A, Gribben J, Alcorn M, Pearson C, McVickers I, Hann IM, Reid C, Wardle D, Gravett PJ, Bacigalupo A Graft rejection following HLA matched T-lympho-

cyte depleted bone marrow transplantation Br J Haematol 63 221, 1986

5 Martin PJ, Hansen JA, Buckner CD, Sanders JE, Deeg HJ, Stewart P, Appelbaum FR, Clift R, Fefer A, Witherspoon RP, Kennedy MS, Sullivan KM, Flournoy N, Storb R, Thomas DE Effects of in vitro depletion of T cells in HLA-identical allogeneic marrow grafts Blood 66 664, 1985

6 Martin PJ, Hansen JA, Torok-Storb B, Durnam D Przepiorka D, O'Quigley J, Sanders J, Sullivan KM, Witherspoon RP, Deeg HJ, Appelbaum FR, Stewart P Weiden P, Doney K, Buckner CD, Clift R, Storb R, Thomas DE Graft failure in patients receiving T cell depleted HLA-identical allogeneic marrow transplants Bone Marrow Transplant 3 445, 1988

7 Fleischhauer K, Kernan NA, O'Reilly RJ, Dupont B, Yang SY Bone marrow allograft rejection by T lymphocytes recognizing a single amino acid difference in HLA-B44 N Engl J Med 323 1818, 1990

8 Kernan NA, Flomenberg N, Dupont B, O'Reilly RJ Graft rejection in recipients of T cell depleted HLA-nonidentical marrow transplants for leukemia Transplantation 43 842, 1987

9 O'Reilly RJ, Kernan NA, Brochstein J, Dinsmore R, Kirkpatrick D, Siena S, Keever C, Jordan B, Shank B, Wolf L, Dupont B, Reisner Y Transplantation of marrow-depleted T cells by soybean lectin agglutination and E-rosette depletion Major histocompatibility complex related graft resistance in leukemic transplant recipients Transplant Proc 17 455, 1985

10 Voogt PJ, Fibbe WE, Marijt WAF, Goulmy E, Veenhof WFJ, Hamilton M, Brand A, Zwaan FE, Willemze R, van Rood JJ, Falkenburg JHF Rejection of bone marrow graft by recipient derived cytotoxic T lymphocytes against minor histocompatibility antigens Lancet 335 131, 1990

11 Perreault C, Decary F, Brochu S, Gyger M, Belanger R, Roy D Minor histocompatibility antigens Blood 76 1269, 1990

12 Goulmy E Minor histocompatibility antigens in man and their role in transplantation Transplant Rev 2 29, 1988

13 Voogt PJ, Goulmy E, Fibbe WE, Veenhof WFJ, Brand A, Falkenburg JHF Minor histocompatibility antigen HY is expressed on human hematopoietic progenitor cells J Clin Invest 82 906, 1988

14 Voogt PJ, Goulmy E, Veenhof WFJ, Hamilton M, Fibbe WE, van Rood JJ, Falkenburg JHF Cellularly defined minor histocompatibility antigens are differentially expressed on human hematopoietic progenitor cells J Exp Med 168 2337, 1988

15 Voogt PJ, Fibbe WE, Veenhof WFJ, Brand A, Goulmy E, van Rood JJ, Falkenburg JHF Cell-mediated lysis of human hematopoietic progenitor cells Leukemia 1 427, 1987

16 Falkenburg JHF, Goselink HM, van der Harst D, van Luxemburg-Heyst SAP, Kooy-Winkelaar YMC, Faber LM, de Kroon J, Brand A, Fibbe WE, Willemze R, Goulmy E Growth inhibition of clonogenic leukemic precursor cells by minor histocompatibility antigen-specific cytotoxic T lymphocytes J Exp Med 174 27, 1991

17 Marijt WAF, Veenhof WFJ, Brand A, Goulmy E, Fibbe WE, Willemze R, van Rood JJ, Falkenburg JHF Minor histocompatibility antigen-specific cytotoxic T cell lines, capable of lysing human hematopoietic progenitor cells, can be generated in vitro by stimulation with HLA-identical bone marrow cells J Exp Med 173 101, 1991

18 van Els CACM, D'Amaro J, Pool J, Blokland E, Bakker A, van Elsen PJ, van Rood JJ, Goulmy E Immunogenetics of human histocompatibility antigens Their polymorphism and immunodominance Immunogenetics 35 161, 1992

19 Fong TAT, Mosmann TR Alloreactive murine CD8+ T cell clones secrete the Th1 pattern of cytokines J Immunol 144 1744, 1990

20 Bacchetta R, de Waal Malefijt R, Yssel H, Abrams J, de Vries JE, Spits H, Roncarolo MG Host-reactive CD4+ and CD8+ T cell clones isolated from a human chimera produce IL-5, IL-2, IFN-gamma, and GM-CSF but not IL-4 J Immunol 144 902, 1992

21 Mangan KF, Zeevi A, Duquesnoy R, Shaddock RK, Gewirtz AM A functional analysis of hematopoietic growth factor production from class I and class II human alloreactive T cell clones Int J Cell Cloning 6 324, 1988

22 Morita CT, Verma S, Aparicio P, Martinez AC, Spits H, Brenner MB Functionally distinct subsets of human gamma/delta T cells Eur J Immunol 21 2999, 1991

23 von Melchner H, Bartlett PF Mechanisms of early allogeneic marrow graft rejection Immunol Rev 71 31, 1983

24 Podack ER, Hengartner H, Lichtenheld MG A central role of perforin in cytolysis? Annu Rev Immunol 9 129, 1991

25 Krahenbuhl O, Tschopp J Perforin-induced pore formation Immunol Today 12 399, 1991

26 Berke G Lymphocyte-triggered internal target disintegration Immunol Today 12 396, 1991

27 Vinci G, Vernant JP, Cordonnier C, Bracq C, Rochant H, Breton-Gorius J, Vainchenker W HLA-DR restriction in suppression of hematopoiesis by T cells from allogeneic bone marrow transplants J Immunol 136 3225, 1986

28 Bunjes D, Heit W, Arnold R, Schmeiser T, Wiesneth M, Carbonell F, Portzsolt F, Rachavachar A, Heimpel H Evidence for the involvement of host-derived OKT-8 positive T cells in the rejection of T-depleted, HLA-identical bone marrow grafts Transplantation 43 501, 1987

29 Goulmy E HLA-A -B restriction of cytotoxic T cells, in Ferrone S, Solheim BG (eds) HLA Typing Methodology and Clinical Aspects New York, NY, CRC, 1982, p 105

30 Madsen M, Johnson HE A methodological study of E rosette formation using AET-treated sheep red blood cells J Immunol Methods 27 61, 1979

31 van der Harst D, Goulmy E, Falkenburg JHF, Kooy-Winkelaar YMC, Luxemburg-Heyst SAP, Goselink HM, Brand A Recognition of minor histocompatibility antigens on lymphocytic and myeloid leukemic cells (submitted)

32 de Bueger M, Bakker A, van Rood JJ, van der Woude F, Goulmy E Tissue distribution of human minor histocompatibility antigens J Immunol 149 1788, 1992

33 Mollison PL, Engelfriet CP, Contreras M ABO, Lewis, Ii, and P groups, in Mollison PL, Engelfriet CP, Contreras M (eds) Blood Transfusion in Clinical Medicine Oxford, UK, Blackwell Scientific, 1987, p 267

34 Degliantoni G, Murphy M, Kobayashi M, Francis MK, Perussia B, Trinchieri G Natural killer (NK) cell-derived hematopoietic colony-inhibiting activity and NK cytotoxic factor J Exp Med 162 1512, 1985

35 Hermann F, Schmidt RE, Ritz J, Griffin JD In vitro regulation of human hematopoiesis by natural killer cells Analysis at a clonal level Blood 69 246, 1987

36 Toretsky JA, Shahidi NT, Finlay JL Effects of recombinant human interferon gamma on hematopoietic progenitor cell growth Exp Hematol 14 182, 1986

37 Souza LM, Boone TC, Gabrilove J, Lai PH, Zsebo KM, Murdock DC, Chazin VR, Bruzewski J, Lu H, Chen KK, Barendt J, Platzer E, Moore MAS, Mertelsman R, Welte K Recombinant human granulocyte colony-stimulating factor Effects on normal and leukemic myeloid cells Science 232 61, 1986

38 Sieff CA, Emerson SG, Donaheu RE, Nathan DG, Wang EA, Wong GG, Clark SC Human recombinant granulocyte-macrophage colony-stimulating factor Multilineage hematopoietin Science 230 1171, 1985

39 Suda T, Suda J, Ogawa M, Ihle JN Permissive role of inter-

leukin 3 (IL-3) in proliferation and differentiation of multipotential hematopoietic progenitors in culture J Cell Physiol 124 182, 1985

40 Clutterbuck EJ, Hirst EMA, Sanderson CJ Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures Comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF Blood 73 1504, 1989

41 Jansen JH, Fibbe WE, Willemze R, Kluin-Nelemans JC Interleukin-4 A regulatory protein Blut 60 269, 1990

42 Bordignon C, Keever CA, Small TN, Flomenberg N, Dupont B, O'Reilly RJ, Kernan NA Graft failure after T cell depleted human leukocyte antigen identical marrow transplants for leukemia II In vitro analysis of host effector mechanisms Blood 74 2237, 1989

43 Martin PJ Increased disparity for minor histocompatibility antigens as a potential cause of increased GVHD risk in marrow transplantation from unrelated donors compared with related donors Bone Marrow Transplant 8 217, 1991

44 Marijt WAF, Veenhof WFJ, Goulmy E, Kluck PMC, Brand A, Willemze R, van Rood JJ, Falkenburg JHF Multiple minor histocompatibility antigen disparities between a recipient and four HLA-identical potential sibling donors for bone marrow transplantation Hum Immunol (in press)