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## Preliminary report

# *In vitro* separation of host specific graft-versus-host and graft-versus-leukemia cytotoxic T cell activities

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**Summary:**

**The association of graft-versus-host disease (GVHD) with lower relapse rates following allogeneic bone marrow transplantation (BMT) in humans led us to analyse post HLA-identical BMT derived anti-host cytotoxic T cells (CTL) for their putative anti-leukemic activity. To establish whether graft-versus-host (GVH) and graft-versus-leukemia (GVL) activities are separate, CTL lines were generated at different time points post-BMT from three patients suffering from acute GVHD. These CTL lines, which exhibited lysis of host normal lymphocytes and neoplastic cells, were analysed at the clonal level. Three functionally different types of clones were characterized: clones directed at host specific minor Histocompatibility (mH) antigens which are shared by patient's peripheral blood lymphocytes (PBL) and leukemic cells; clones recognizing only host PBL but not host leukemic cells; and putative GVL clones directed at patient's neoplastic cells only. These data could explain the long controversies on dissection of GVH and GVL activities. Our results demonstrate that GVH and GVL activities can be dissected, while non-separable effector cells which exhibit both activities do exist as well.**

One of the major obstacles in allogeneic bone marrow transplantation (BMT) is the occurrence of graft-versus-host disease (GVHD).[1] T cell depletion of the donor bone marrow inoculum shows a reduction in the incidence and severity of GVHD but an increase in relapse rate. Mature T cells in this donor bone marrow inoculum vital for graft acceptance and responsible for GVHD are probably also needed to eliminate residual leukemic cells: the graft-versus-leukemia (GVL) effect.[2,3] A substantial number of experimental animal models indicate a GVL effect of allogeneic BMT.[4,5] Although controversial, mouse data support the notion that the GVL reaction can be distinguished from the GVH reactivities. Bortin *et al.*[6] were the first to demonstrate that GVL reaction could be induced by alloimmunization while avoiding GVHD. In humans several clinical trials have suggested a direct relationship between the GVL effect and acute and chronic GVHD.[7,8] However, clinical data from Butturini *et al.*[3] and Horowitz *et al.*[2] support a GVL effect independent of GVHD that is altered by T cell depletion. To our knowledge, the only *in vitro* result in humans demonstrating that the GVL and GVHD effect may at least partially be separable, is the isolation of cytolytic T cells from normal donors recognizing allogeneic leukemic cells.[9,10]

We analysed post-BMT derived CTL lines from three patients transplanted for acute lymphoblastic T cell leukemia to ascertain whether separate populations of T cells in the bone marrow inoculum are responsible for the anti-host and anti-leukemic activities.

## Patients and methods

Three patients received bone marrow grafts from their HLA identical siblings. Acute GVHD was noted in all three patients. Blood samples were collected from the recipient at the time neoplastic cells were present, pre-BMT, at different time points post-BMT, and from the healthy sibling donor. CTL lines were generated as described earlier in detail.[11] Briefly, post-BMT cells (i.e. donor derived) are stimulated with patient's pre-BMT PBL. The CTL lines were further expanded by weekly stimulation with recipient pre-BMT EBV-lymphoblastoid cell lines (EBV-LCL) and donor PBL. Cytotoxic activity of the T cell lines and clones was tested in a standard $^{51}Cr$ release assay at different effector/target cell ratios. As target cells we used phytohaemagglutinin stimulated PBL (i.e. PHA blasts) of donor and recipient, EBV-LCL of donor and recipient, and patient's leukemic cells (thawed and preincubated overnight in RPMI 1640 supplemented with 15% human serum). Specific lysis was calculated as described.[11] The CTL lines were analysed for expression of CD4, CD8 and the NK markers CD16 and CD56. Cloning, with or without CD4 depletion

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(i e sorting the CD8 positive population), of the CTL lines at 100, 30, 10, 3 and 1 cell/well was carried out After reasonable growth was obtained, each individual clonal population was assayed for cytotoxicity on recipient PHA blasts and leukemic cells All positive clones were further expanded enabling broader functional analyses.

## Results

Figure 1 shows that post-BMT derived CTL lines from patients 1 and 2 lysed both normal and neoplastic cells of the host as early as day 35 after BMT, while from patient 3, such CTL lines were generated from day 100 after BMT In all three cases, the lysis was found to be patient specific as no lysis was observed when donor cells were used as target cells Only in the case of patient 3 some EBV specific lysis became apparent

Subsequently, one CTL line of each patient was used for cloning the CTL line of day 100, 35 and 100 post-BMT from patients 1, 2 and 3 respectively These CTL lines are negative for the NK markers CD16 and CD56 and do not lyse any of the NK sensitive target cell lines Daudi, HL60 or K562 (data not shown) Cloning at different seeding concentrations per well of the CTL lines was repeated at least twice Table 1, which is representative of a series of experiments, shows that independent of the number of cells seeded per well, three functionally different types of clones can be isolated from each of the three patients' CTL lines First, a large number of 'dual' positive clones eliminating both patient's normal and neoplastic cells were found Second, clones directed at host PBL only, and third, host leukemic specific clones were isolated Further expansion of these clones yielded stable 'dual' positive and 'single' anti-host directed clones The host specific leukemia directed clones appeared, however, to be unstable and seemed to lose their activity in time in culture

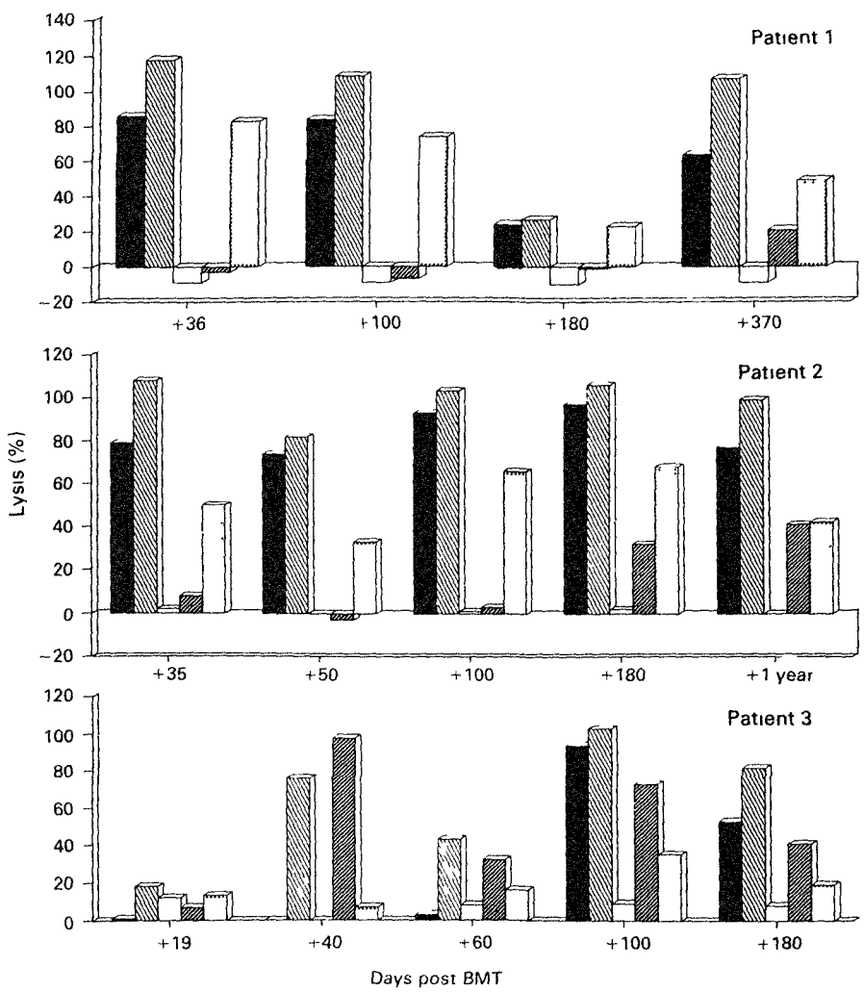


**Figure 1** Cytotoxicity pattern of post-BMT derived CTL lines of patients 1 2 and 3 (■) host PHA (▨) host EBV-LCL (□) donor PHA (▨) donor EBV-LCL ( ) host leukemia

**Table 1** Cytolytic patterns of GVH and GVL specific T cell clones

| | | *Target cells* | |
|---|---|---|---|
| | | *PHA blast* | *Leukemic cells* |
| *Patient 1* | | | |
| 100 c/w[a] | 100P1[b] | 90[c] | 94 |
| | 100P23 | 74 | 0 |
| | 100L1 | 0 | 59 |
| 30 c/w | 30P12 | 80 | 89 |
| | 30P3 | 58 | 0 |
| | 30L29 | 4 | 89 |
| *Patient 2* | | | |
| 10 c/w | D1 | 95 | 48 |
| | H9 | 89 | 1 |
| | L15 | 3 | 31 |
| 3 c/w | D2 | 99 | 40 |
| | H1 | 95 | 2 |
| | L4 | 2 | 24 |
| *Patient 3* | | | |
| 0.3 c/w | FA46 | 38 | 28 |
| | FA43 | 41 | 14 |
| | GA22 | 11 | 44 |

[a]Number of cells plated per well
[b]Clone designation
[c]Percentage specific lysis

## Discussion

Our data clearly show, by analysis of post-BMT lymphocytes from three patients, that effector cells, potentially representing GVH and GVL activities, can be identified *in vitro*. These activities are displayed by T cells since no NK-like characteristics were observed. The possibility of induced LAK activity is almost negligible because of our culture conditions. An explanation for the controversies in dissecting GVH and GVL activities is that not only GVH and GVL specific clones were isolated but also clones reactive with ligands shared by host PBL and leukemic cells. Characterization of these 'dual' functional clones, directed at host specific mH antigens which are expressed on both patient's PBL and leukemic cells, is in agreement with recent observations in our laboratory.[12] Because we did not test our clones for their clonality at a molecular level, it is possible that the last type of clones are a summation of clones displaying lysis on neoplastic and normal patient's cells. On the other hand, our *in vitro* analysis is compatible with results obtained in the mouse. Truitt *et al.*[13] described separate as well as overlapping GVL and GVH reactive cell populations. At present we are aiming to obtain stable GVL clones, in order to establish whether these clones show unique or broad anti-leukemic activity, and whether they are directed at mH antigens with limited tissue distribution or against truly leukemia associated antigens. The fact that we are able to isolate single GVL directed T cell clones may contribute to a further understanding of possible effector cells of GVL, and hopefully may lead to new approaches for potentiating anti-leukemic reactivity without inducing severe GVHD.

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## References

1. BORTIN MM. Acute graft-versus-host disease following bone marrow transplantation in humans: prognostic factors. *Transplant Proc* 1987, **19:** 2655–2657.
2. HOROWITZ MM, GALE RP, SONDEL PM *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990, **75:** 555–562.
3. BUTTURINI A, BORTIN MM, GALE RP. Graft-versus-leukemia following bone marrow transplantation. *Bone Marrow Transplant* 1987, **2:** 233–242.
4. ALEXANDER P. Treatment of acute leukaemia by whole body irradiation and marrow transplantation: 25 years from mouse to man. *Br J Cancer* 1981, **43:** 713–714.
5. OKUNEWICK JP, MEREDITH RP. *Graft Versus Leukemia in Man and Animal Models.* CRC Press: Boca Raton, FL 1981
6. BORTIN MM, TRUITT RL, RIMM AA, BACH FH. Graft-versus-leukaemia reactivity induced by alloimmunisation without augmentation of graft-versus-host reactivity. *Nature* 1979, **281:** 490–491.
7. WEIDEN PL, FLOURNOY N, THOMAS ED *et al.* Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *New Engl J Med* 1979, **300:** 1068–1073.
8. WEIDEN PL, SULLIVAN KM, FLOURNOY N, STORB R, THOMAS ED. Antileukemic effect of chronic graft-versus-host disease. Contribution to improved survival after allogeneic marrow transplantation. *New Engl J Med* 1981, **304:** 1529–1532.
9. SOSMAN JA, OETTEL KR, SMITH SD, HANK JA, FISCH P, SONDEL PM. Specific recognition of human leukemic cells by allogeneic T cells: II. Evidence for HLA-D restricted determinants on leukemic cells that are crossreactive with determinants present on unrelated nonleukemic cells. *Blood* 1990, **75:** 2005–2016.
10. SLAVIN S, ACKERSTEIN A, NAPARSTEK E, OR R, WEISS L. Hypothesis. The graft-versus-leukemia (GVL) phenomenon: is GVL separable from GVHD? *Bone Marrow Transplant* 1990, **6:** 155–161.
11. GOULMY E. Minor histocompatibility antigens in man and their role in transplantation. *Transplant Rev* 1988, **2:** 29–53.
12. VAN DER HARST D, GOULMY E, FALKENBURG JHF *et al.* Recognition of minor histocompatibility antigens on lymphocytic and myeloid leukemic cells by cytotoxic T cell clones. (Submitted for publication).
13. TRUITT RL, SHIH C, LEFEVER AV, TEMPELIS LD, ANDREANI M, BORTIN MM. Characterization of alloimmunization-induced T lymphocytes reactive against AKR leukemia in vitro and correlation with graft-vs-leukemia activity in vivo. *J Immunol* 1983, **131:** 2050–2058.