Endogenously Generated Activated Killer Cells Circulate After Autologous and Allogeneic Marrow Transplantation but Not After Chemotherapy


After marrow transplantation, major histocompatibility complex (MHC)-unrestricted natural killer (NK) lymphocytes are among the first cells to appear in the circulation. Activated killer cells are among the first cells to appear in the circulation. If the donor marrow has first been depleted of T cells to prevent graft-versus-host disease (GvHD), these regenerating cytotoxic cells are activated, spontaneously producing interleukin-2 (IL-2), gamma interferon (IFN), and tumor necrosis factor (TNF) and may have a significant role as a primary defense against viral reactivation and in the elimination of residual host malignancy. We studied 43 patients with hematologic malignancy, treated by allogeneic TD-BMT, autologous nondepleted BMT, or chemotherapy alone to investigate (a) the mechanisms underlying the generation of these activated killer cells, (b) the range of conditions under which they are produced, and (c) their surface phenotype. We showed that IFN-secreting activated killer cells with the capacity to kill MHC-nonidentical NK-resistant targets are generated 4 to 6 weeks after either allogeneic TD-BMT or autologous BMT but do not appear after treatment with chemotherapy. Production therefore is not owing to T-cell depletion per se or to host blocking such interactions with anti-MHC class-I monoclonal antibody (MoAb) treatment of target cells or by CD8 MoAb treatment of effector lymphocytes. The results show that activated CD16+ CD3- effector cells are present after both allogeneic and autologous BMT but not after chemotherapy alone and that these cells kill targets independently of recognition of class-I MHC molecules.

NATURAL KILLER (NK) lymphocytes are among the first cells to recover after allogeneic bone marrow transplantation (BMT).1,2 If the donor marrow has first been depleted of T cells to prevent graft-versus-host disease (GvHD), these regenerating cytotoxic cells are activated, spontaneously producing interleukin-2 (IL-2), gamma interferon (IFN), and tumor necrosis factor (TNF)4,5 and behaving like cells with lymphokine-activated killer (LAK) activity with the ability to kill major histocompatibility complex (MHC) nonidentical virus-infected and malignant target cells not susceptible to NK activity.6,8 They respond to exogenous IL-2 with a further enhancement of cytokine production7 and a further increase in cytokine effector function6,12 and may be able to make a significant contribution to antiviral and antineoplastic host defenses. The mechanisms by which these activated cells are generated is not clear, nor is it known whether the cells are unique to allogeneic T-lymphocyte-depleted bone marrow transplantation (TD-BMT) or whether they are produced in other conditions in which marrow regeneration occurs (eg, after autologous BMT or after intensive chemotherapy for hematologic malignancy).

To study these issues we investigated whether cytokine-secreting LAK-like cells are produced either after autografting with marrow containing T cells or after chemotherapy for hematologic malignancy. Cells with LAK activity generated from normal individuals may be CD16+ CD3- or CD16- CD3+13-18 and the two populations may have a different tissue distribution18 and repertoire of target cell recognition.16,17 We therefore examined the phenotype of endogenously generated LAK-like cells after BMT to determine whether both these lineages are represented. Finally, we investigated whether the ability of endogenously generated cells with LAK activity to kill MHC nonidentical targets is due to recognition of allogeneic class I MHC molecules19 by blocking such interactions with anti-MHC class-I monoclonal antibody (MoAb) treatment of target cells or by CD8 MoAb treatment of effector lymphocytes. The results show that activated CD16+ CD3- and CD16+ CD3+ effector cells are present after both allogeneic and autologous BMT but not after chemotherapy alone and that these cells kill targets independently of recognition of class-I MHC molecules.

MATERIALS AND METHODS

Forty-three patients with hematologic malignancy were studied; 22 received HLA-matched sibling allografts. Their ages ranged from 10 to 45 years (median 22 years). Ten patients received transplants for acute myeloid leukemia (AML) (three in second remission), six for common acute lymphoblastic leukemia (cALL) (three in second or subsequent remission), four for T-acute lymphoblastic leukemia (T-ALL), and two for chronic granulocytic leukemia (CGL) in first chronic phase. Recipients in first remission or first chronic phase were conditioned with cyclophosphamide 60 mg/kg x 2 plus Mesna; those in second or subsequent remission or relapse received cyclophosphamide 45 mg/kg x 2 and cytosine arabinoside 3 g/m2 twice daily for three days. All patients received...
single-dose total body irradiation (TBI) at a maximum prescribed dose of 750 cGy to the lungs at a dose rate of 15 cGy/min with a mean received midplane dose of 752 cGy. Marrows were treated with the Royal Free T-cell depletion protocol using IgM mouse MoAbs RFT12 (CD6) and RFT8 (CD8) in association with two rounds of rabbit complement.5 Five patients received marrow that had also been purged with RFT2 (CD7). Median time taken to reach 1 x 10^9/L leukocytes in peripheral blood of recipients was 21 (range 15 to 34) days. All grafts in the group studied were self-sustaining. Mean T cells infused were 0.78 x 10^9/kg, representing a mean T-cell depletion of 96.6%. No patient received routine GvHD prophylaxis posttransplant. Fifteen patients had no GvHD, and five had grade 1 to 2 GvHD that responded to short-course oral methyl prednisolone.

Twelve patients received autografts of cryopreserved marrow harvested in remission. In eight patients with AML, the marrow was otherwise unmanipulated: In four with cALL it was purged prior to preservation with pre-B-cell MoAbs (common ALL antigen; cAL-LA)-CD10/SB4-CD20 at 100 µg/mL marrow concentrate; total 5 mg in 50 mL) together with rabbit C (25 mL). The age of the autograft recipients ranged from 14 to 58 years (median 34 years), and the patients were conditioned with cyclophosphamide and thioguanine (DAT 3 + chemotherapy for AML with daunorubicin, cytosine arabinoside, and of antibodies used are given in the Materials and Methods section. PBMs were enumerated on an Ortho ELT 800 WS.

Viral infections. Viral infections posttransplant were defined on the basis of detection of viral antigen expression and subsequent viral isolation following inoculation of throat swabs, urine, or blood into appropriate tissue culture cells.

Two patients receiving allografts (UPNs 175 and 180) developed cytomegalovirus (CMV) reactivation detected in throat swabs and urine, respectively, on weekly surveillance cultures, as did two autograft recipients (UPNs 521 and 519). Herpes simplex (HSV)-seropositive recipients were treated with prophylactic acyclovir, and only one (allograft) recipient reactivated HSV. Patients showing evidence of active viral infection were excluded from subsequent analyses.

Preparation of mononuclear cells. With Ethical Practices Committee approval, venous blood (60 to 120 mL) was drawn from recipients on one to four occasions 3 to 6 weeks (depending on peripheral WBC count) posttransplant/chemotherapy. After centrifugation on Ficoll and washing in RPMI 1640 medium (Flow), the mononuclear cells were depleted of plastic-adherence monocytes on Nunc tissue culture plates (GIBCO), resuspended in RPMI 1640 medium supplemented with 2 mmol/L glutamine, penicillin 100 IU/mL, streptomycin 100 µg/mL (GIBCO), and 10% vol/vol fetal calf serum (FCS, Sera Laboratories, Crawley, England) and used in cytotoxic assays.

Elimination of lymphocyte subpopulations. Washed cells were incubated for 30 minutes on ice with CD3 (OKT3) (Ortho, High Wycombe, England) or CD16 (Leu-11b) MoAb as described previously,22 at saturating concentration. The cells were spun, and a dilution (1:3) of rabbit complement (C') was added. The cells were incubated for 40 minutes at 37°C. A second round of C' lysis was performed after washing. The degree of depletion was then determined by immunofluorescence analysis (Table I). For blocking experiments, CD8 (RFT8) and CD3 (OKT3) MoAb and W6/32, an anti-class-I MHC antibody,24 were used at 2 x cytotoxic saturating concentrations which had previously produced maximal inhibition of two cytotoxic T cell clones.5 In these experiments, complement was omitted. Reagents of the Leu series were obtained from Becton Dickinson (Cowley, England); RFT8 was provided by Professor George Janossy and W6/32 was a gift from Corlab Research, Cambridge, England.

Surface marker phenotype. The surface antigen phenotype of the cells was determined by two-color immunofluorescence in the microtiter plate system,23 and staining was evaluated under an epi-immunofluorescence microscope. Cells were incubated with the specific murine anti-human MoAbs as first layer; after the cells were washed, staining was visualized by fluorescein or rhodamine-conjugated goat anti-mouse isotype-specific F(ab), reagents (Southern Biotechnology, Birmingham, AL) in 500 to 1,000 cells. The following MoAbs were used: Leu-4, OKT3 (CD3; pan-T); RFT8 (CD8; T-suppressor/cytotoxic) and Leu-11b (CD16; NK cells). Target cells and labeling. The target cell for the LAK assay was an Epstein-Barr virus (EBV)-transformed B-lymphoblastoid cell line (LCL) established from the peripheral blood of a normal donor as described previously.2 The cell line was maintained in suspension culture in supplemented RPMI 1640 medium (Flow). B-LCLs were labeled by incubation with 51Cr (Na,CrO4) for 140 minutes. As a control, killing of the NK target, 51Cr-labeled K562, was measured in parallel. Finally, thawed cryopreserved AML blast cells were used to detect MHC-unrestricted killing of leukemic target cells by activated effector lymphocytes. These targets were prepared and labeled as previously described.13

Cytotoxicity assays. Full details of the methodology of these assays were described previously.2 One hundred-microliter volumes of target cell suspension at 5 x 10^5 cells/mL were dispersed into V-shaped microtitre plate wells (GIBCO). The effector cells were then added in a series of effector-target (E/T) ratios each in

### Table 1. Percentage Composition of PBMs

<table>
<thead>
<tr>
<th></th>
<th>Normal Subjects (n = 14)</th>
<th>Allograft BM Recipient (n = 15)</th>
<th>Autograft BM Recipient (n = 11)</th>
<th>Chemotherapy (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PBMs (x 10^9/L)</td>
<td>2.61 ± 0.35</td>
<td>1.12 ± 0.17*</td>
<td>0.90 ± 0.16*</td>
<td>1.08 ± 0.17*</td>
</tr>
<tr>
<td>CD3</td>
<td>71 ± 9</td>
<td>27 ± 11*</td>
<td>56 ± 5</td>
<td>49 ± 6†</td>
</tr>
<tr>
<td>CD4</td>
<td>52 ± 11</td>
<td>11 ± 4*</td>
<td>15 ± 5*</td>
<td>17 ± 5†</td>
</tr>
<tr>
<td>CD8</td>
<td>31 ± 7</td>
<td>29 ± 8</td>
<td>38 ± 9</td>
<td>26 ± 6</td>
</tr>
<tr>
<td>CD16</td>
<td>11 ± 3</td>
<td>27 ± 3†</td>
<td>15 ± 2.5</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>CD25</td>
<td>&lt;1</td>
<td>9 ± 3 (n = 8)†</td>
<td>3 ± 1 (n = 9)†</td>
<td>4 ± 1 (n = 8)†</td>
</tr>
<tr>
<td>CD3 subsets</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3^* CD16^+</td>
<td>2 ± 1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>CD3^* Leu-19^+</td>
<td>3 ± 1</td>
<td>1 ± 1 (n = 4)</td>
<td>1 ± 1 (n = 4)</td>
<td>2 ± 1 (n = 4)</td>
</tr>
</tbody>
</table>

PBM numbers and phenotype at 4 to 6 weeks after auto/allo-TD-BMT or chemotherapy. Values are mean ± SEM. Details of patients in each group and of antibodies used are given in the Materials and Methods section. PBMs were enumerated on an Ortho ELT 800 WS.

*P < .01; †P < .05; ‡P < .001 significant differences from normal by multi t testing.
triplicate. The assays were conducted for four hours, after which half of the supernatant was removed and counted for isotope release. The percentage of specific lysis for each target was calculated as follows: experimental release - spontaneous release/maximum release - spontaneous release % 100%, where the maximum release was that observed with target cells exposed to 1% sodium dodecyl sulfate (SDS, Sigma, Poole, England). Spontaneous release from LCL was <15% of the maximum release; from K562, spontaneous release was <10% of maximum.

Peripheral blood mononuclear cell (PBMC) culture for γ-IFN production. PBMs were suspended at 2 x 10^6 cells/mL in serum-free medium and cultured for 48 hours. Supernatants were filtered through a 0.22-μm membrane (GIBCO) and stored at -70°C prior to testing.

γ-IFN assay. γ-IFN was measured with a commercially available radioimmunoassay (RIA,Sucrose IRMA Boots-Celtech Diagnostics, Slough, England). The minimum sensitivity of the assay was 1 U/mL γ-IFN.

Statistical analyses. Data or log-transformed data were analyzed by parametric methodology including paired and unpaired t-testing and multi-t testing for analysis of variance (ANOVA). The Rubycrate Beebstat program (London) running on a BBC Master computer was used for statistical analyses.

RESULTS

Phenotype and cytotoxic activity of PBMs. Table 1 shows the absolute numbers and phenotypic composition of PBMs in normal controls and in the three patient groups. The percentage of CD4⁺ cells was low in all patient groups. CD3⁺ cells were significantly reduced in allograft and chemotherapy recipients only, whereas CD16⁺ cells were a significantly greater than normal proportion of PBMs in the allograft recipients. The percentage of CD25 (IL-2R)-bearing cells was significantly higher in both allograft and chemotherapy patients, but remained <10% of the total number of cells. Leu-19⁺ cells, in contrast, were not significantly different from normal (mean + 4) in any patient group, nor was there any increase in the proportion of Leu-19⁺ cells that were also CD3⁺ (Table 1). Less than 2% of cells were CD3⁻ CD16⁺ in the patients.

Figure 1 shows the function of the PBMs. Figure 1A compares killing of the NK cell target K562; Fig 1B shows killing of the LAK target EBV-transformed LCLs. Effector lymphocytes were from (a) normal donors, (b) patients who received unmanipulated autografts following chemotherapy and TBI, (c) patients who received allogeneic TD-BMT, or (d) patients who were recovering from chemotherapy as treatment for AML.

Killing of K562 by allograft recipients was greater than normal, whereas the cytotoxicity of cells from autograft recipients was not significantly different from normal. However, killing of K562 by cells from chemotherapy patients was significantly impaired (Fig 1A).

As previously reported,2,12 EBV-LCLs are not susceptible to NK activity; thus, they were not killed by lymphocytes from any of the normal donors (Fig 1B). However, LCLs are vulnerable to killing by lymphocytes from all patients who have received TD-BMT within the preceding 6 weeks and also to killing by lymphocytes obtained from all recipients of unmanipulated autografts (Fig 1B). In contrast, lymphocytes from patients recovering from chemotherapy alone are not active against LCLs, even when the lymphocytes were obtained at the same time posttherapy as the activated cells from allo/autograft recipients. Killing of allogeneic LCLs declines by background by 3 to 4 months after both allogeneic2,11,12 or autologous (data not shown) BMT. Allograft recipients also kill autologous (donor-type) EBV-transformed LCLs.12 In autograft recipients, successful EBV transformation of lymphocytes could not be achieved regularly pretransplant, presumably because of the consequences of prior exposure to intensive chemotherapy. In two autograft patients in whom EBV transformation was achieved pre-BMT, autologous effector cells obtained after the BMT produced a Cr⁵¹ release of 12% and 17% at an E/T ratio of 20:1.

Investigation of MHC-unrestricted killing of cryopreserved myeloid leukemia blast cells in the four-hour Cr⁵¹ release assay showed that levels of isotope release were low in all patient groups (Fig 1C).

Secretion of γ-IFN. Lymphocytes from allogeneic TD-BMT recipients spontaneously secrete cytokines, including γ-IFN, for the first 3 months after the procedure, representing further evidence of cell activation.6,7 Figure 2 shows that lymphocytes from autograft recipients also spontaneously secrete γ-IFN to levels not significantly different from those of allograft recipients. Again, lymphocytes from normal donors and from patients receiving chemotherapy are not activated by this criterion (Fig 2).

Allostimulation by blood products. To discover whether differences in activation between BMT recipients and patients recovering from chemotherapy alone were associated with major differences in the amount or nature of allostimulation from blood products received in the early (< 4 weeks) posttreatment period, the number of products received during this time was determined for all patients in each group. Table 2 shows that all groups received substantial blood product support and that although autologous BMT recipients received significantly greater support than chemotherapy patients, there was no significant difference between patients treated with chemotherapy or allogeneic BMT. In addition, regression analyses of the number of units of platelets/blood transfused vs LAK activity in BMT recipients (Cr⁵¹ release from LCL at E/T 20:1) showed no correlation.

Role of viral infection. Surveillance cultures for reactivation of herpes group viruses revealed three episodes of activation in allograft recipients and two in autograft patients (described in the Materials and Methods section). These patients were excluded from subsequent analyses; levels of killing obtained immediately before viral reactivation were above the mean but below the maximum for each group (27.5%, 26.0%, and 29% for allograft at an E/T ratio of 20:1 and 22% and 25% for autograft patients).

Phenotype of cells with cytotoxic effector function. Activated MHC-unrestricted cytotoxicity may be mediated by CD3⁺ CD16⁺ or CD3⁺ CD16⁻ cells,13-18 both of which are present after autologous and allogeneic BMT (Table 1). To determine whether activated killing of virus-infected targets was exclusively the province of either of these populations or whether it was mediated by CD8⁺ cytotoxic cells,19 we

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Fig 1. Killing of K562 (NK target A) and EBV-transformed lymphoblastoid cell line (LAK target B) at three different E/T ratios. Data are the mean ± SEM for four different groups of individuals, normal donors (n = 14), autologous transplant recipients (n = 8), allogeneic marrow recipients (n = 17) and chemotherapy patients (n = 8). Details of patient groups and of assay are given in the Materials and Methods section. Analysis of variance showed that killing of K562 by autograft recipients was not significantly different from normal, but that killing by chemotherapy patients was significantly reduced at E/T ratios of 40:1 (P < .03) and 20:1 (P < .006). Killing by allograft recipients was increased at E/T ratios of 40:1 (P < .03) and 20:1 (P < .04). (B) Killing of EBV-LCL was significantly greater than normal in allograft and autograft recipients at all E/T ratios (P < .001) but there was no difference between transplant groups or between normal individuals and chemotherapy patients. (C) Killing of cryopreserved allogeneic myeloid blast cells by allograft (n = 5), autograft (n = 5), and chemotherapy patients (n = 6) at an E/T ratio of 20:1, 50:1, and 80:1. Results are shown for each individual studied. Normal donors (n = 5) produced <1% Cr release at any ratio. Because of the low values, statistical analyses for significant differences are not performed.
CIRCULATION OF NK CELLS

Figure 2. Units (expressed as log_{10}) γ-IFN released from PBM's cultured in vitro, as described in the Materials and Methods section. Cells were obtained from normal subjects (n = 9) or from allograft recipients (n = 9), autograft recipients (n = 9), or patients receiving chemotherapy alone (n = 8) all at 3 to 6 weeks after the procedure. Each bar represents data from a single individual and is the mean of duplicate radioimmunometric assays.

Analysis of variance showed highly significant difference (P < .001) between BMT recipients and normal individuals/patients receiving chemotherapy, but no difference between autologous and allogeneic BMT or between normal individuals and chemotherapy patients.

Selective depletion of each subset. Figure 3 shows that CD8 depletion had little effect. Table 3 shows that LCL killing was not abrogated by removal of CD3+ or CD16+ populations individually, although CD16 depletion, unlike CD3 depletion, did produce a decline in effector function. Depletion of both CD3+ and CD16+ populations reduced cytotoxicity to background, indicating that killing was essentially caused by both CD16+CD3− and CD16−CD3+ cells.

Target recognition by MHC-unrestricted effector cells. The interaction of the CD16+CD3− population with their targets is invariably independent of MHC recognition9−21 but MHC-unrestricted activated killing by CD16+CD3− effector cells may result from their ability to recognize multiple MHC class-I allospecificities.19 To investigate this possibility, four patients showing high residual killing of LCLs after CD16 depletion were studied. PBM's were again depleted of Fc-γIII receptor-positive (CD16) lymphocytes, and MHC-directed interactions were blocked either by treating LCL targets with W6/32, an MoAb binding to nonpolymorphic epitopes of class-I MHC,24 or by treating CD16+ effector cells with anti-CD8 MoAb. Figure 4 shows that neither had any effect on the target killing by the CD16− population.

Figure 3. Cytotoxic effector function against an EBV-LCL in an autograft and an allograft patient before and after CD8+ cell depletion with MoAb RFT8 C'. After lysis, PBM's contained <2% CD8+ cells. Autograft PBM's (--), autograft PBM's after CD8+ lysis (-----), allograft PBM's (---), allograft PBM's after CD8+ lysis (----).

Table 2. Blood Product Support

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Mean No. of Units of Blood (Range)</th>
<th>Mean No. of Units of Platelets (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allograft</td>
<td>5.50 (3-9)</td>
<td>54.75 (12-146)</td>
</tr>
<tr>
<td>Autograft</td>
<td>7.25 (4-10)</td>
<td>79.25 (39-138)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>4.43 (2-7)</td>
<td>30.24 (12-41)</td>
</tr>
</tbody>
</table>

Patients were transfused with packed RBCs or plasma-reduced units to maintain hemoglobin levels >8 g/dL. Platelet support was provided using single-donor platelet concentrates or (in one chemotherapy and one allograft patient) single-donor cell separator packs after the patients became refractory to random donor platelets. ANOVA showed no significant difference in blood or platelet units received between allograft and autograft patients or between allograft and chemotherapy patients. Significant difference existed between autograft and chemotherapy patients for both blood (P = .013) and platelets (P = .007). Details of patients are given in the Materials and Methods section.
Table 3. Effect of Subset Depletion on LAK Activity

<table>
<thead>
<tr>
<th>UPN</th>
<th>Type of Graft</th>
<th>Cytotoxic v LCL*</th>
<th>Percentage Control Killing After Depletion of</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>Allo</td>
<td>11</td>
<td>63 125</td>
</tr>
<tr>
<td>171</td>
<td>Allo</td>
<td>14</td>
<td>41 170 &lt;10</td>
</tr>
<tr>
<td>172</td>
<td>Allo</td>
<td>42</td>
<td>57</td>
</tr>
<tr>
<td>204</td>
<td>Auto</td>
<td>41</td>
<td>72</td>
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<td>177</td>
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<tr>
<td>180</td>
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<td>21</td>
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</tr>
<tr>
<td>527</td>
<td>Auto</td>
<td>34</td>
<td>63</td>
</tr>
</tbody>
</table>

*Cytoxicity quoted at E/T ratio of 20:1 on an EBV-positive lymphoblasted cell line.
†Depletion experiments were performed as described in Materials and Methods section. Residual CD16+ cells <1%. Residual CD3+ cells <2%. E/T ratios are quoted at 20:1.

DISCUSSION

We investigated the circumstances under which marrow recovery generates cells with LAK-like activity with the ability to kill NK-resistant target cells in an MHC-unrestricted manner. Activated killing was evident after TD-BMT2,6,12 (Fig 1B) and also after autografting with marrow in which T cells remained (Fig 1B). Thus, induction does not require either alloreactivity between donor and host or removal of T cells from the donor graft. Because lymphocytes from both transplant groups also spontaneously secrete cytokines, including γ-IFN (Fig 2), activation has an autocrine/paracrine component.25 This population of cells with LAK-like activity may be important after BMT in suppressing viral reactivation and for elimination of residual malignant cells either by direct cytotoxicity or by secretion of inhibitory cytokines.10,26,37 Endogenous LAK cells are not produced after chemotherapy alone. Therefore, it is unlikely that activation is a consequence of exposure to the alloantigenic stimulus of blood products, because the chemotherapy patients received blood product support to a level not significantly different from that of the allograft recipients. In addition, regression analyses of the level of blood product support of degree of LAK activity showed no correlation. More likely, activation in BMT recipients reflects the immune disregulation that follows reconstitution of a host, treated with ablative chemotherapy and TBI, with an incomplete “immune network,” derived initially only from cells present in the peripheral blood and marrow compartments. Immune regulation appears to be restored 3 to 4 months after BMT, since activation of cytotoxic effector cells has then disappeared.2,6,11 Cytokine-secreting activated killer cells are not always detected after conventional allogeneic BMT even though the same disregulation would be predicted to exist.28,29 In these recipients, activation may be reduced by the immunosuppressive effects of agents [such as cyclosporin A (CSA)] given as GvHD prophylaxis. Indeed, if recipients of allogeneic TD-BMT receive CSA, activation, measured by spontaneous cytokine production, is inhibited.28

LAK effectors after BMT may be either CD16+CD3− or CD16−CD3+. The observation that both CD16+ and CD3+ cells may have LAK activity is consistent with clonal analyses showing that LAK cells may be generated from both sets of progenitors13,14,30,31 and that cells of either phenotype may have MHC-unrestricted effector function.32,33 However, these same clonal analyses suggest that CD3+CD16− LAK cells occur at a substantially lower frequency than their CD16+ counterparts. Moreover, LAK cells generated in short-term culture ex vivo or after infusion of IL-2 at supraphysiologic doses, are almost all in the CD16−CD3− fraction.13,14,32,33 These results appear to conflict with our observations after TD/auto-BMT when significant (albeit reduced) LAK-like activity remains in the CD16− population and is abolished by subsequent treatment with CD3 MoAb and C. This apparent contradiction may well reflect the way in which the cellular basis for the LAK phenomenon is modified by lymphokine dose and exposure duration.36 The high cytokine levels and relatively short exposure times generally used for ex vivo or infusion studies appear to favor production of CD16+ LAK cells, whereas more prolonged exposure to the relatively low levels of cytokines attained physiologically (eg, after BMT) may preferentially expand CD3+ LAK-like cells.13,14,36 In addition, the phenotype of the
endogenous LAK cells produced may be dependent on the precise phenotypic profile of the cells available for recruitment \cite{13,14,18,19} and thus may be modified by different marrow-purging techniques.\cite{11} If the repertoire of target cell recognition and the pattern of tissue distribution are nonidentical in the two subpopulations,\cite{14,18} it may be optimal for the BMT recipient to regenerate both CD16\textsuperscript{+}CD3\textsuperscript{−} and CD3\textsuperscript{−}CD16\textsuperscript{−} activated killer cells.

Two other subsets of cells may be important in CD3\textsuperscript{−}-mediated MHC-unrestricted cytotoxicity, CD16\textsuperscript{+}CD3\textsuperscript{−} and Leu-19\textsuperscript{+}CD3\textsuperscript{−} cells. However, after BMT, CD16\textsuperscript{+}CD3\textsuperscript{−} cells form <2% of PBMs whereas Leu-19\textsuperscript{+}CD3\textsuperscript{−} cells represent 0% to 1% of the total PBM count (Table 1). This low proportion together with our depletion data make it unlikely that either subset make a major contribution to the endogenous LAK activity observed after BMT, although clonal analyses of cells from normal donors show that lymphocytes with these phenotypes may have potent LAK function.\cite{77}

Most BMT patients had no evidence of viral reactivation in the early post-BMT period (discussed in the Materials and Methods section). Nonetheless, the presence of CD16\textsuperscript{+}CD3\textsuperscript{−} MHC-unrestricted cytotoxic effector cells raises the possibility that endogenous LAK cells are produced after BMT as a response to reexpression of latent viruses during the immediate posttransplant period, since viral infection of normal individuals may lead to development of MHC-unrestricted effector cells with an identical CD16\textsuperscript{+}CD3\textsuperscript{−} phenotype.\cite{19,38} However, loss of MHC restriction in the context of viral infection appears to result from production of MHC class I-alloreactive and CD8\textsuperscript{+}CD3\textsuperscript{−} T cells. Thus, MHC-"unrestricted" cytotoxicity during infectious mononucleosis can be blocked by treatment of allo-
geneic target cells with anti-HLA class I MoAb (W6/32) or of effector lymphocytes by CD8 MoAb.\cite{19} In contrast, blocking experiments with these MoAbs after BMT (Fig 4) show that CD3\textsuperscript{+}CD16\textsuperscript{−}-dependent cytotoxicity is independent of interaction with MHC molecules and depletion of CD8\textsuperscript{+} cells does not abolish MHC-unrestricted killing (Fig 3).

Use of autologous or allogeneic (TD-) BMT for treatment of acute leukemia and lymphoma produces a higher proportion of long-term survivors and a substantially lower risk of relapse\cite{10,40,42} than chemotherapy alone. Although the higher doses of chemoradiation that can be used before the BMT may contribute much to this improvement, the observation that activated killer cells are generated after both autologous and TD allogeneic BMT but not after chemotherapy alone is consistent with the suggestion that cells with LAK activity may be important in elimination of minimal residual malignancy.\cite{10,12} Antileukemic activity may be mediated predominantly by release of cytokines inhibitory to leukemia progenitor cell growth\cite{19} rather than by direct cytotoxicity detectable in a four-hour Cr\textsuperscript{51} release assay (Fig 1C). If activated killer cells are important in elimination of residual leukemia progenitor cells, administration of agents such as IL-2 that induce or enhance LAK activity directed against virus-infected or malignant cells\cite{6,34} would be predicted to reduce morbidity/mortality from virus reactivation and from relapse of malignant disease in patients treated either by chemotherapy or by BMT.

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