ABSTRACT  Previous studies suggested that natural killer (NK) cells are involved in the regulation of the growth and differentiation of pluriptotent hematopoietic stem cells. To establish whether the effector cells responsible for the in vivo resistance to bone marrow (BM) transplants and the in vitro inhibition of colony-forming units (CFU) may represent identical or overlapping populations, we used a rat system for syngeneic BM transplantation, with and without the transfer of large numbers of peripheral blood large granular lymphocytes (LGLs). BM reconstitution was measured by the in vivo formation of syngeneic CFU in the spleen (CFU-s). Because of the very low frequency of CFU-s in normal rat BM, we fractionated BM cells in Percoll density gradients, which provided a 2- to 5-fold enrichment in CFU-s in the lower-density fractions. Although these fractions contained <10% of the total cells, they contained >75% of the CFU-s and allowed for the transfer of significantly fewer donor cells. At the time of BM transplantation, radiation-resistant asialoganglioside GM1-positive LGLs, with high NK activity, accounted for a significant percentage of the lymphoid cells in the irradiated recipient. The in vitro regulatory role of these cells on engraftment was demonstrated by their depletion (by i.v. injection of small amounts of anti-asialo-GM1 antiserum before BM transplantation), which resulted in a significant increase in the number of CFU-s. Conversely, a 50% inhibition in CFU-s was found when CFU-s-enriched BM fractions were preincubated in vitro with LGLs. Additional experiments, involving selective in vivo depletion of NK cells followed by LGL repopulation, directly demonstrated the involvement of LGLs in the regulation and growth of syngeneic pluriptotent hematopoietic stem cells. Our results further support the hypothesis that LGLs are involved directly or via humoral factors in the homeostasis and regulation of hematopoietic stem cell growth and differentiation.

The pioneering findings that natural killer (NK) cells displayed reactivity against a subpopulation of normal thymus cells (1, 2) led to the hypothesis that these natural effectors could be involved not only in immune surveillance against transformed cells (3) but also in the regulation or differentiation or proliferation of normal cells, particularly early stem cells of the hematopoietic system. Subsequently, an appreciable number of studies have been carried out to further address this issue. In the mouse, a series of positive correlations have been described between the phenomenon of resistance to F1 and allogeneic hematopoietic grafts regulated by the hybrid histocompatibility (Hh) genes and the NK system (4). Further insight into the role of NK activity in the control of allogeneic stem cell growth and differentiation was suggested by the work of Warner and Dennert (5), who were able to adoptively impart bone marrow (BM) resistance to nonresistant hosts by using a cloned T-cell line with NK activity. In addition, the destruction of allogeneic BM cells in vivo by NK cells has also been observed in experiments in which the clearance of 125I-uridine-labeled BM cells after intravenous inoculation was increased by interferon-inducing agents (6).

However, because of the paucity of specific NK-cell markers and the difficulties in isolating large numbers of highly purified NK effector cells for adoptive transfer in the mouse, the question of whether NK cells and the effector cells of natural resistance are the same or related cells still remains open. In the human, purified large granular lymphocytes (LGLs), the cells associated with NK activity (7), have been shown to inhibit in vitro granulopoiesis (8, 9) and in vitro erythropoiesis (10, 11). Although these data suggest a role for NK cells in the normal physiologic and pathologic regulation of syngeneic BM stem-cell growth and differentiation, the in vivo significance of these in vitro findings is not clear.

We therefore have performed studies with rats, since large numbers of LGLs can be readily isolated (12) and tested for their effects on the growth and differentiation of syngeneic BM cells. The present report describes a series of experiments using an anti-asialoganglioside GM1 (anti-asGM1) protocol similar to the one previously used to examine the direct role of LGLs in the clearance of tumor cells from the lungs (13) and in the inhibition of tumor metastasis (14). The present results provide direct evidence for in vivo inhibition of growth or differentiation of syngeneic pluriptotent hematopoietic stem cells (PHSCs) by LGLs.

MATERIALS AND METHODS

Animal and Tumor Cell Lines. All experiments were performed with 4- to 6-week-old male or female Fischer (F344) inbred rats. Rats to be transplanted with BM were given 900–1200 rads (~400 R/min; 1 rad = 0.01 Gy; 1 roentgen (R) = 0.258 mC/kg) of gamma radiation from a model 68A MARK I 137Cs irradiator (J. L. Shepherd, Glenoble, CA) prior to the treatments indicated in Results. The mouse lymphoma cell line YAC-1 was maintained in suspension culture in RPMI 1640 medium supplemented with 10% fetal bovine serum (Biofluids, Rockville, MD), 100 units of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM glutamine.

Preparation of Cell Suspensions. Spleen cell suspensions were prepared as described (13). Peripheral blood mononu-

Abbreviations: asGM1, asialo-ganglioside GM1; BM, bone marrow; CFU, colony-forming units; CFU-s, spleen CFU; LGL, large granular lymphocyte; MHC, major histocompatibility complex; NK, natural killer; NRS, normal rabbit serum; PBL, peripheral blood leukocyte; PHSC, pluriptotent hematopoietic stem cell.

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clear leukocytes (PBLs) were obtained by separation from heparinized whole blood on Ficoll/Hypaque, washed with Hanks’ balanced salts solution (HBSS), and resuspended in complete medium. Adherent cells were removed by incubation on plastic dishes followed by adherence on nylon-wool columns. Highly enriched populations of LGLs (80–90%) and T cells (95–98%) from nonadherent PBLs were obtained by centrifugation in a discontinuous density gradient of Percoll (Pharmacia Chemicals, Uppsala, Sweden) (12). Morphologic differential cell counts were determined by inspection of Giemsa-stained Cytocentrifuge slides. Suspensions of LGLs or T cells (5 × 10⁶) were injected i.v. in 0.5 ml of HBSS 2 hr before BM transplantation.

BM cell suspensions were prepared aseptically from the femurs of syngeneic 6- to 8-week-old F344 inbred rats. The cell suspension was washed in HBSS and layered onto Ficoll/Hypaque gradients to remove cellular debris, red cells, and mature granulocytes. Further fractionation was then achieved on Percoll density gradients as reported in Results. The desired number of cells from each Percoll fraction or whole BM was then adjusted to 0.5 ml in HBSS for i.v. inoculation.

CFU-s Assay. The CFU-s (spleen colony-forming-unit) assay was performed 9 days after BM transplantation as previously described (15). BM recipients were housed under laminar-flow conditions and given acidified water.

In Vivo Treatment with Anti-asGM1 Serum. Anti-asGM1 (Wako Chemicals, Dallas, TX) was diluted in phosphate-buffered isotonic saline and filtered through a 0.22-µm filter (Millipore) before injection i.v. (0.5 ml per rat). Control rats were injected with the same volume of normal rabbit serum (NRS).

In Vitro Assay for NK Activity. Various concentrations of effector cells were incubated with 10⁶-5¹⁵Cr-labeled YAC-1 or ¹¹¹In-oxine-labeled BM (16) target cells for 4 hr or 18 hr at 37°C in round-bottomed, 96-well microtiter plates as described (12). All groups were tested in triplicate. Standard errors were always <5% of the mean and are not always included in the tables.

Proliferation Assay. Proliferation of BM fractions was assessed by incorporation of [methyl-3H]thymidine (1 µCi, specific activity 6.7 mCi/mM, New England Nuclear; 1 Ci = 37 GBq) by 10⁵ cells during an 18-hr incubation.

Cell-Cycle Analysis. The percentage of cells in stages G₁ and S was analyzed by total DNA quantitation, using a propidium iodoide binding assay with subsequent analysis on an ortho-Cytograph (Ortho Instruments).

Statistical Analysis. The data for the CFU-s assay were analyzed for statistical significance between groups (five to eight animals per group) by a one-tailed Student’s t test or the Mann–Whitney U test. Results from representative experiments are presented, although all experiments were repeated, with similar results, at least five times.

RESULTS

Effect of Lethal Irradiation on Splenic NK Activity. According to the results previously reported (15), lethal irradiation is necessary to condition the splenic microenvironment of animals undergoing BM transplantation. Irradiation also depresses NK activity (17), but it is unclear whether this is due to a decrease in the number of LGLs, an inactivation of these cells, or a suppression of their activity by radiation-induced suppressor cells. To address this question, we analyzed LGLs from rats given lethal irradiation (Table 1). Twelve to 24 hr after irradiation, the total number of spleen cells and NK cytotoxicity were markedly diminished compared to the controls receiving sham treatment. Although the total number of LGLs per spleen was reduced by a factor of ∼10, the proportion of LGLs among the remaining cells much exceeded that seen in untreated controls (43% vs. 4%). When the whole spleen cell suspensions were separated on Ficoll/Hypaque gradients to remove high-density cells and cellular debris, the cytotoxic activity from irradiated spleen cells was significantly increased, even to the point of being greater than the activity from sham controls (38% vs. 25%). However, as observed previously (12), there is not a direct correlation between NK activity and LGL percentage. These results confirm the loss of NK activity in unseparated spleen cell preparations following lethal whole-body irradiation. However, the data also demonstrate substantial residual NK activity in Ficoll/Hypaque-prepared cells, and an increase in the proportion of LGLs in the spleen despite the decrease in total NK activity and total number of LGLs.

Enrichment of Rat PHSCs by Use of Discontinuous Percoll Gradients. The frequency of PHSCs in adult rat BM, as measured in the CFU-s assay, is extremely low (18). In order to examine, in vitro, the direct effects of highly purified LGLs on the PHSCs, it was important to enrich the BM cell population for the PHSCs before testing them as NK targets. To do this, BM cells were separated on Percoll density gradients and tested for characteristics of stem cells. Table 2 displays some characteristics of five different BM fractions obtained from Percoll gradients, with densities ranging from 43.3% to 61.6% (vol/vol). According to these data, we were able to isolate >10% of the BM cells (fractions 1 and 2) that were highly proliferative as shown by thymidine incorporation and cell-cycle analysis.

These same cells showed a 2- to 5-fold increase in CFU-s activity when injected into irradiated recipients. These data suggest that these PHSC-enriched populations might be more useful than whole BM in the further analysis of the susceptibility of BM cells to NK activity.

Cytotoxic Effect of LGLs on BM Fractions. Until now the hypothesis that NK cells can kill BM stem cells has been supported primarily by circumstantial evidence. In fact, direct cytotoxicity against BM cells has been difficult to show, particularly in a syngeneic situation, probably due to a very low frequency of susceptible target cells in the whole BM population. With the enrichment of PHSC activity on Percoll gradients it seemed possible to more sensitively assess the cytotoxic activity of LGLs against BM stem-cell targets. In Table 3 are shown the results of direct cytotoxicity of freshly isolated PBLs, LGLs, and T cells against syngeneic (F344) whole or fractionated BM target cells. Since a minimum of 12 hr was required to achieve significant lysis (data not shown), all target cells were radiolabeled with ¹¹¹In-oxine (16) to minimize the spontaneous release of radioactivity in the assay (<0.1%/hr). In these experiments, the cytotoxicity of 10⁶ PBLs against the syngeneic whole BM population used as targets was negligible (1.9%). However, when low-density syngeneic BM cells enriched in PHSCs were used as targets, the amount of cytotoxicity increased considerably (8.1%). This increase in susceptibility was not seen with the higher-density fraction-5 cells devoid of PHSC activity.

Table 1. Analysis of spleen cell suspensions 24 hr after whole-body irradiation

<table>
<thead>
<tr>
<th>Spleen group</th>
<th>Cells per spleen, % LGL</th>
<th>% lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated</td>
<td>80.0 4 3.2 16.0</td>
<td>16.0 ± 1</td>
</tr>
<tr>
<td>Ficoll/Hypaque</td>
<td>40.0 8 3.2 25.0</td>
<td>25.0 ± 2</td>
</tr>
<tr>
<td>Irradiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated</td>
<td>1.0 43 0.4 6.0</td>
<td>6.0 ± 1</td>
</tr>
<tr>
<td>Ficoll/Hypaque</td>
<td>0.7 42 0.3 38.4 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

*Data reflect cytotoxicity (effector/target cell ratio 50:1) against YAC-1 target cells.
Table 2. Analysis of Percoll fractionation of rat bone marrow

<table>
<thead>
<tr>
<th>Fraction</th>
<th>% Percoll (vol/vol)</th>
<th>% cell recovery</th>
<th>[3H]Thymidine, cpm per 10^6 cells</th>
<th>% G1+S cells</th>
<th>CFU-s per spleen*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43.3</td>
<td>1</td>
<td>11,328 ± 1,333†</td>
<td>22</td>
<td>&gt;50 (confluent)†</td>
</tr>
<tr>
<td>2</td>
<td>49.1</td>
<td>10</td>
<td>18,460 ± 1,056†</td>
<td>27</td>
<td>&gt;50 (confluent)‡</td>
</tr>
<tr>
<td>3</td>
<td>54.1</td>
<td>38</td>
<td>7,683 ± 770†</td>
<td>14</td>
<td>4 (1–8)</td>
</tr>
<tr>
<td>4</td>
<td>61.6</td>
<td>46</td>
<td>1,165 ± 81†</td>
<td>5</td>
<td>4 (4–6)</td>
</tr>
<tr>
<td>Pellet</td>
<td>—</td>
<td>3</td>
<td>414 ± 105</td>
<td>9</td>
<td>19 (18–30)</td>
</tr>
<tr>
<td>FH</td>
<td>—</td>
<td>100</td>
<td>3,638 ± 217</td>
<td>NT</td>
<td>21 (17–33)</td>
</tr>
</tbody>
</table>

One million cells from the indicated Percoll gradient fraction or from Ficoll/Hypaque (FH) were injected per rat. NT, not tested.

*Median (range).
†Significantly greater than FH control (P < 0.001).
‡Significantly greater than control (P < 0.001).

(3.1%). To determine the nature of the effector cell, highly purified LGLs and T cells were used as effectors, and a high level of cytotoxicity was obtained only with the LGLs. These results showed that the enrichment of both LGL effectors and low-density BM target cells is necessary to observe significant levels of direct cytotoxicity.

To directly determine whether the cytotoxicity observed in the syngeneic combination reflected an effect on the PHSCs in the BM, we measured the CFU-s activity of the fraction-2 Percoll-enriched BM cells after incubation with LGLs or T cells (Fig. 1). Percoll-enriched BM cells were cultured overnight with syngeneic LGLs or T cells at a 1:10 ratio before being injected into lethally irradiated syngeneic recipients. In these experiments there was a consistent decrease in the number of detectable CFU-s-derived colonies in the fraction-2 BM cells upon culture with LGLs, compared to the same fraction-2 cells incubated overnight in medium alone. Although less than seen with LGLs, significant inhibition was also found when BM cells were incubated with purified, unstimulated, high-density cells, which were mainly T cells.

Effect of Anti-asGM1 and CFU-s Activity. The above results are consistent with the hypothesis that radiation-resistant NK cells could play an important role in the destruction of syngeneic PHSCs following BM transplantation. To directly test this hypothesis, a series of experiments was undertaken to evaluate the role of the radiosensitive asGM1-positive LGLs in inhibiting the formation of CFU-s following BM transfer (Fig. 2). To selectively delete LGL and NK activity, lethally irradiated rats were injected i.v. with serial dilutions of anti-asGM1 2–6 hr after irradiation and 24 hr prior to undergoing syngeneic BM transplantation. We previously showed (14) that in vivo treatment of rats with low doses of anti-asGM1 antiserum (1:40–1:100 dilutions) was effective in selectively deleting NK activity and circulating LGLs without affecting T-cell or macrophage functions. The present results showed a dose-dependent decrease in NK activity and increase in the number of CFU-s in the anti-asGM1-treated groups compared to the NRS-treated controls. The in vivo treatment of BM cells with anti-asGM1 did not affect the ability of these cells to form colonies either in vivo (CFU-s) or in vitro (CFU-c) (data not shown), ruling out the possibility of a direct interaction of the anti-asGM1 antiserum on BM progenitors.

In Vivo, Inhibition of CFU-s by LGLs. The enhanced colonization of CFU-s in rats pretreated with small amounts of anti-asGM1 suggested an in vivo role for asGM1-positive NK cells. Although the anti-asGM1 antibody was shown not to have a direct effect on the BM progenitors and the doses of antibody used were previously shown to be selective for NK cells (14), it was still possible that the effects of the anti-asGM1 could have been via some other cell population or mechanism. To directly examine the role of LGLs in the control of CFU-s proliferation, we performed a series of experiments to determine the effects of selective reconstitution of NK activity, by adoptive transfer of highly purified LGLs into NK-depleted BM recipients (Fig. 3). In these experiments, irradiated rats received one i.v. injection of anti-asGM1 (1:80 dilution) 3–6 hr after irradiation. Twenty-four hours later, these recipients were repopulated with 5 × 10^6 LGLs or T cells, followed by 10^6 fraction-2 BM cells. As was shown in Fig. 2, the number of spleen colonies in anti-asGM1-treated recipients was increased by almost 100% compared to the NRS control. The adoptive transfer of a relatively low number of LGLs significantly restored the ability of these recipients to inhibit the formation of CFU-s. When T cells were injected into anti-asGM1-treated recipients, a slight decrease in spleen colonies was observed, in agreement with our previous results of coculture in which we obtained some diminished CFU-s proliferation when BM cells were incubated with enriched T cells (Fig. 1). The mechanism of this inhibition is unknown but could relate to the contamination of the high-density fraction with some

Table 3. Susceptibility of BM fractions to lysis by LGLs and T cells

<table>
<thead>
<tr>
<th>F344 effector cells</th>
<th>Cytotoxicity against F344 BM target cells, % lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>BM Fraction 2</td>
</tr>
<tr>
<td>PBLs</td>
<td>50:1</td>
</tr>
<tr>
<td>LGLs</td>
<td>25:1</td>
</tr>
<tr>
<td>T cells</td>
<td>25:1</td>
</tr>
</tbody>
</table>

Cytotoxicity was measured in 18-hr assays using the indicated effector/target (E/T) ratios. Values differing significantly from control (medium in place of effector cells) are indicated by * (P < 0.05) and † (P < 0.01).
are in control. P
rat). P
1:80
spleen (±SD)
transplantation. Additionally, experimental to
direct evidence highly purified stem
of the
cells these
in
FIG. 2. Effect of anti-asGM1 (αASGM1) treatment of irradiated
cell s on PBL. NK activity and on the formation of CFU-s.
Antiserum (various dilutions) or NRS (1:40 dilution) was given i.v.
(0.5 ml) 2–6 hr after irradiation and 24 hr prior to the injection of 10^6
Ficoll/Hypaque BM cells. NK activity was monitored from PBLs 24
hr after anti-asGM1 injection. Values represent either CFU-s per
spleen (±SD) or % cytotoxicity (±SD) in a ^{51}Cr-release assay with
YAC-1 cells as targets. P values for CFU-s are in comparison to
NRS-treated controls (NS, not significant).

LGLs (<3%); however, the absence of direct cytotoxicity by
these cells (Table 3) suggests that this inhibition is more likely
due to a different mechanism.

DISCUSSION
The experimental model described here was designed to
provide direct evidence for the in vivo role of LGLs in the
control of the growth and development of syngeneic BM-
derived stem cells in irradiated recipients. Our in vivo results
with highly purified rat LGLs and Percoll-enriched BM stem
cells as targets clearly demonstrated an important role for
LGLs in the inhibition of CFU-s following syngeneic BM
transplantation. Additionally, in vitro cytotoxicity studies
strongly suggested that this inhibition is at least partially
due to the cytotoxic activity of LGLs for enriched BM stem
cells.

Previous in vitro studies suggested that human NK cells
could suppress autologous and allogeneic erythroid colony-
forming and burst-forming units (CFU-E and BFU-E) and

granulocyte/macrophage colony-forming units (CFU-GM) in
culture (8–10). In addition, Degliantoni et al. (19) reported
that multipotent precursors (CFU-GEMM) are also inhibited
by cell populations enriched in NK cells. These studies
provided circumstantial evidence that NK cells play a role in
the regulation of autologous BM stem-cell growth and dif-
erentiation in vivo. Further evidence for this role was
provided by Holmberg et al. (20), who were able to

demonstrate the inhibition of CFU-s-derived colonies in mice by
coculture of murine BM with syngeneic spleen cells with high
NK activity.

The inhibition of autologous or allogeneic BM stem cells by
NK cells could be a direct consequence of either direct cell
contact (killing) of the BM precursors, the release of cyto-
toxic/cytostatic factors, or both. LGLs do secrete a variety
of substances, some cytotoxic [such as NK cytotoxic factor
(21)] and others cytostatic [such as interferon (22)], which
are known to affect hematopoietic progenitors (23, 24). In fact, it
was shown by Degliantoni et al. (25) that NK cells have a
hematopoietic colony-inhibiting activity and that the factor
responsible for this activity is indistinguishable from tumor
necrosis factor.

In contrast to soluble factors from LGLs there is less
evidence for cell–cell contact and the killing of BM targets by
NK cells. BM cells have generally been found to be poor
targets for cytotoxicity by NK cells (26), although spleen
cells proliferating after BM transplantation were found to be

good competitive inhibitors in an NK assay against typical
NK-sensitive target cells (27). In the present experiments,
low levels of killing were observed against whole BM.
However, the levels of killing were significantly increased by
the use of enriched (Percoll gradient) stem-cell targets and
further increased with purified LGL effector cells but not T
cells. Moreover, as shown in Fig. 1, the coculture of LGLs
with stem-cell-enriched BM always resulted in an inhibition
of spleen colonies. The present results are consistent with the
hypothosis that there is a direct correlation between the in
vitro cytotoxicity by LGLs and the subsequent inhibition of
BM CFU in the spleen. However, direct proof of this corre-
lation is still lacking.

An important prerequisite to the contention that NK cells
play an important role in the inhibition of syngeneic BM
growth and differentiation in irradiated recipients is the
demonstration of substantial numbers of residual NK-active
cells following irradiation. Our results showing a decrease in
the total number of LGLs but an increase in the percent LGL and
NK activity per 10^7 cells (Table 1) are consistent with the
presence after irradiation of a residual high concentration of
very active LGLs. Consistent with a role for these cells in the
in vivo resistance to BM transplantation (28), anti-asGM1-
treated recipients depleted of NK activity had substantially
increased numbers of CFU-s following syngeneic BM trans-
fer (Fig. 2). As previously noted (12), the percentage of LGLs
does not always exactly correlate with levels of NK activity.
This discrepancy probably reflects the fact that not all LGLs
are NK cells and not that cells other than LGLs have NK
activity.

The critical role of LGLs in this system was shown by the
adoptive transfer of LGLs into anti-asGM1-treated rats,
resulting in a reconstitution of NK activity and inhibition of
syngeneic CFU-s formation to 80% of control levels (Fig. 3).
These data are very similar to our recent reports of a
reconstitution of NK activity (13) and in vivo clearance of
tumor cells and inhibition of tumor metastasis by LGLs in
anti-asGM1-treated rats (14).

We can now speculate that asGM1-positive LGLs, with NK
activity, which account for a large percentage of cells in the
irradiated spleen microenvironment, influence the anti-BM
response in syngeneic recipients by inhibiting either the seeding
or proliferation of adoptively transferred syngeneic BM stem
cells. Although we do not have sufficient data to distinguish these two mechanisms, we do know that in vitro treatment of BM cells with anti-AsGM1 antiserum (with or without complement) does not alter the number of CFU-s (data not shown). These results, along with the reconstitution of the anti-BM response by LGLs, demonstrate that the observed results are not due to a direct effect of anti-AsGM1 on the seeding and/or proliferation of BM stem cells in the spleen.

The present data do not exclude the potential role of other cells in controlling the growth of syngeneic BM transplants. In fact, in all of our experiments we observed a marginal but consistent effect of T cells on reducing the number of CFU-s observed. These results are similar to the effects seen on the clearance of tumor cells (13) or inhibition of tumor cell metastases (14). Since the transfer of $5 \times 10^6$ LGLs is required for a detectable effect on the formation of CFU-s (data not shown), these results probably reflect an additional effect of T cells in exerting an anti-BM response, as has been suggested from in vitro results (29).

Although the effector cells for NK activity and syngeneic BM graft rejection are very similar, they may reflect different subpopulations of LGLs with different antigenic specificities. It has been proposed that NK cells recognize early differentiation antigens (30), the transferrin receptor (31), laminin (32), or various viral determinants (33). However, it is still unclear how LGLs are able to recognize and kill histocompatible BM stem cells. Daley and Nakamura (34) have shown that first-generation (F1) animals were able to specifically recognize parental BM cells and that this response seemed to reflect the recognition of some major histocompatibility complex (MHC) genes and some non-MHC genes. Our cytotoxicity results using syngeneic BM targets (F344) and an increased cytotoxicity using MHC-identical but allogeneic BM targets (data not shown) are consistent with the observation that allogeneic BM cells are more rapidly eliminated from the circulation than syngeneic BM cells (35). These data further support the hypothesis that NK cells can recognize MHC and non-MHC genes involved in BM graft rejection.

The results presented here demonstrate that LGLs play an important role in inhibiting the growth and/or proliferation of adoptively transferred syngeneic BM stem cells and suggest that the control of recipient NK activity may be an important factor in influencing the outcome of autologous or MHC-matched BM transplants in humans.

We thank Mr. John Wine for his expert technical assistance. T.B. thanks Wakо Chemicals USA, Inc. (Dallas, TX) for partial financial support to attend the 16th International Leucocyte Culture Conference (Cambridge, England), where some of these data were presented.