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Inhibition of pluripotent hematopoietic stem cells of bone marrow by large granular lymphocytes

(natural killer cells)

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ABSTRACT **Previous studies suggested that natural killer (NK) cells are involved in the regulation of the growth and differentiation of pluripotent 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 vivo* 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 pluripotent 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 F_1 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 ^{125}I-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 pluripotent 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 ^{137}Cs 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-

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Abbreviations: asGM1, asialoganglioside GM1; BM, bone marrow; CFU, colony-forming unit(s); CFU-s, spleen CFU; LGL, large granular lymphocyte; MHC, major histocompatibility complex; NK, natural killer; NRS, normal rabbit serum; PBL, peripheral blood leukocyte; PHSC, pluripotent hematopoetic 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 (5×10^6) 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 10^4 ^{51}Cr-labeled YAC-1 or ^{111}In-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*-^{3}H]thymidine (1 μCi, specific activity 6.7 mCi/mM, New England Nuclear; 1 Ci = 37 GBq) by 10^5 cells during an 18-hr incubation.

Cell-Cycle Analysis. The percentage of cells in stages G_1 and S was analyzed by total DNA quantitation, using a propidium iodide binding assay with subsequent analysis on an Ortho Cytofluorograph (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 spleen cells 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 signficantly 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.

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

Spleen group	Cells per spleen, no. × 10^{-6}	% LGL	LGLs per spleen, no. × 10^{-6}	% lysis (±SD)*
Sham treatment				
Unfractionated	80.0	4	3.2	16.0 ± 1
Ficoll/Hypaque	40.0	8	3.2	25.0 ± 2
Irradiation				
Unfractionated	1.0	43	0.4	6.0 ± 1
Ficoll/Hypaque	0.7	42	0.3	38.4 ± 2

*Data represent cytotoxicity (effector/target cell ratio 50:1) against YAC-1 target cells.

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 ^{111}In-oxine (16) to minimize the spontaneous release of radioactivity in the assay (<0.1%/hr). In these experiments, the cytotoxicity of F344 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 2. Analysis of Percoll fractionation of rat bone marrow

Fraction	% Percoll (vol/vol)	% cell recovery	[^{3}H]Thymidine, cpm per 10^5 cells	% G_1+S cells	CFU-s per spleen*
1	43.3	1	11,328 ± 1,333†	22	>50 (confluent)†
2	49.1	10	18,460 ± 1,056†	27	>50 (confluent)†
3	54.1	38	7,683 ± 770†	14	4 (1–8)
4	61.6	46	1,165 ± 81	5	4 (4–6)
Pellet	—	3	414 ± 105	9	19 (18–30)
FH	—	100	3,638 ± 217	NT	21 (17–33)

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 ($P < 0.001$).

(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 show 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 experimets 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 radioresistant 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 depleting 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 vitro* 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.

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

F344 effector cells		Cytotoxicity against F344 BM target cells, % lysis		
Source	E/T	BM	Fraction 2	Fraction 5
PBLs	50:1	1.9 ± 0.5	8.1 ± 1.0*	3.1 ± 0.5
LGLs	25:1	11.8 ± 0.9†	25.8 ± 1.8†	6.0 ± 1.1*
T cells	25:1	0.8 ± 1.0	0.7 ± 1.7	0.1 ± 0.3

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 † ($P < 0.01$).

***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 10^5 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 first 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

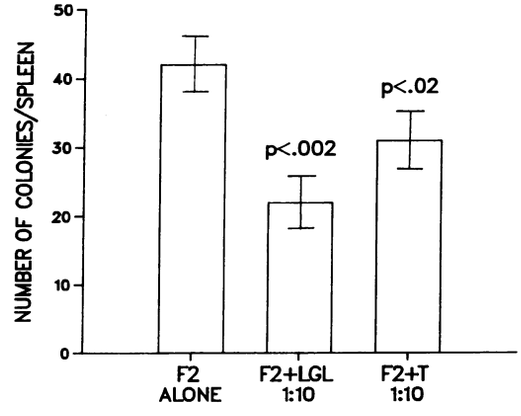

FIG. 1. Inhibition of CFU-s by LGLs and T cells. BM cells from Percoll fraction 2 (F2) were incubated with LGLs or T cells (effector/target ratio 10:1) for 18 hr before transfer into irradiated recipients (10^5 BM F2 cells per rat). Values represent number of CFU-s per spleen (mean ± SD) 9 days after BM transfer. *P* values are in comparison to F2 cells alone.

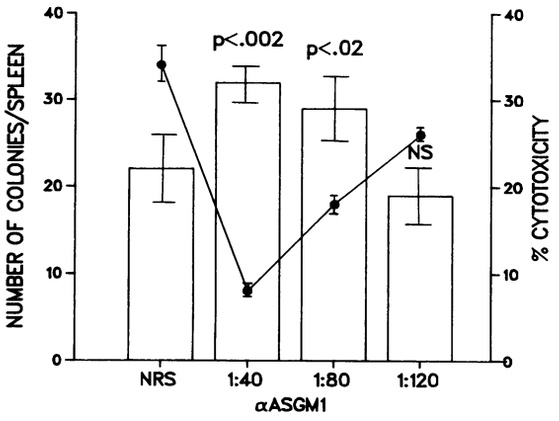

FIG. 2. Effect of anti-asGM1 (αASGM1) treatment of irradiated recipients on PBL NK activity and on the formation of CFU-s. Antiserum (various dilutions) or NRS (1:40 dilution) was given i.v. (0.5 ml) 2–6 hr after irradiation and 24 hr prior to the injection of 10^6 Ficoll/Hypaque BM cells. NK activity 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

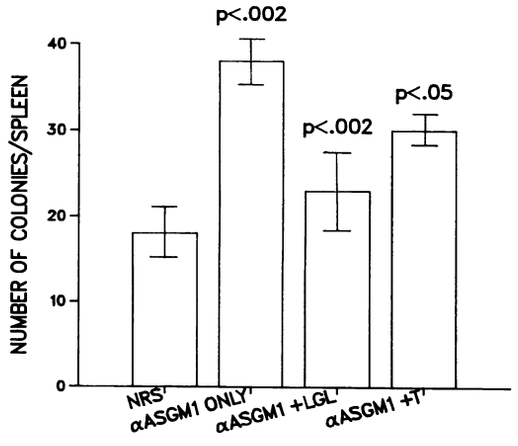

FIG. 3. Inhibition of CFU-s by the adoptive transfer of LGLs or T cells (5×10^6) into irradiated recipients pretreated with anti-asGM1 (αASGM1; 1:80 dilution). Values represent the number of CFU-s per spleen (±SD) 9 days after BM transfer (10^5 BM fraction-2 cells per rat). *P* value for the anti-asGM1-only group is in comparison to NRS control. *P* values for anti-asGM1 + LGLs and anti-asGM1 + T cells are in comparison to the anti-asGM1-only group.

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 differentiation *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 cytotoxic/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 hypothesis 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 correlation 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 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 transfer (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 5×10^6 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 (F_1) 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.

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