Hematopoietic Stem Cell Depletion by Restorative Growth Factor Regimens During Repeated High-Dose Cyclophosphamide Therapy

By Ronald L. Hornung and Dan L. Longo

We studied the effects of six cycles of repeated cyclophosphamide (CTX) therapy followed by restorative therapy with either granulocyte-macrophage colony-stimulating factor (GM-CSF) or G-CSF on the hematopoietic stem cell compartment. Stem cell function was assessed by serially transferring bone marrow cells from CTX-CSF-treated mice into lethally irradiated recipient mice. Bone marrow cells from mice that initially received either G-CSF or GM-CSF after CTX therapy more rapidly lost the ability to repopulate the recipient lymphoid organs, showed a dramatic loss of hematopoietic progenitors, a more rapid loss of CFU-S capacity, and a 40% to 50% reduction in marrow repopulating ability (MRA). Interleukin-1 (IL-1) appeared to have little effect on the CTX-treated mice when used alone. However, when administered before the CTX-CSF regimen, IL-1 prevented the stem cell depletion as determined by CFU-C, CFU-S, and MRA through the serial transplantation procedures. These results support the hypothesis that repeated treatments with myelosuppressive drugs followed by stimulation with the CSFs may induce damage to the host stem cell compartment, and further suggest that pretreatment with IL-1 before CTX therapy may prevent this stem cell damage.

This is a US government work. There are no restrictions on its use.

MATERIALS AND METHODS

Mice. Female C57BL/6 mice, whose leukocytes express either the Lyt 5.1 or Lyt 5.2 leucocyte marker on their surface, were obtained from the Animal Production Facility, NCI-Frederick Cancer Research and Development Center (Frederick, MD), housed in a specific-pathogen free animal facility, and routinely used at 7 to 10 weeks of age.

Drugs and reagents. The following agents were used in this study: recombinant murine GM-CSF with a specific activity of 4 × 10^7 U/mg and recombinant human IL-1α with a specific activity of 1.4 × 10^9 U/mg were generously supplied by Immunex Corporation (Seattle, WA), recombinant human G-CSF with a specific material and colony-forming units-culture (CFU-C) activity. It was our belief that as we serially transferred the original Lyt 5.2 stem cells from the drug-treated mice, the cells should eventually lose their MRA. It is the different rates at which stem cells from these different groups of mice lose MRA through serial transfer that will enable us to determine if supportive cytokine therapy may have reduced the stem cell function of these mice.
activity of 1 × 10^8 U/mg was kindly supplied by Amgen (Oakland, CA). CTX was purchased from Aldrich Chemical Co (Milwaukee, WI). The antimus mouse Lyt 5.1 and Lyt 5.2 IgG2a monoclonal antibodies were purchased from New England Nuclear (Boston, MA). The fluorescein isothiocyanate (FITC)-labeled goat antimus IgG2a antibody was purchased from FisherBiotech (Orangeburg, NY).

CTX and cytokine administration schedule. A six-cycle drugcytokerine regimen was administered to groups of C57BL/6 Lyt 5.2 mice as shown in Fig 1. IL-1 (0.1 µg/mouse) was delivered intraperiortially (i.p.) on day 1 of the cycle to the appropriate groups of mice. CTX (350 mg/kg) was administered i.p. to all but the control group of mice on the second day of each cycle. Although this dose of CTX was not lethal as a single dose, it is the maximum tolerable single dose and did represent a considerable insult to the hematopoietic system of these mice. Six repeated cycles of this dose of CTX was lethal to 50% of the mice (an LD50). On days 3 through 6 of each cycle, mice were administered a single 1 µg/mouse i.p. injection of either G-CSF or GM-CSF. On days 8 through 14 of each cycle, mice were rested before starting the next round of therapy, which was repeated five more times. In all there were seven groups of mice in this study. The control group received no therapy, while the second group received only CTX therapy. The third group received only IL-1 pretreatment with CTX therapy, while the next two groups were administered either G- or GM-CSF restorative therapy after CTX. The last two groups received CTX therapy sandwiched between IL-1 pretreatment and G- or GM-CSF restorative therapy. After the last therapy cycle, mice were allowed to recover for 7 weeks before the start of the serial transfer phase.

Serial transfer procedures and determination of marrow repopulating ability. The serial transfer phase, diagrammed in Fig 2, was initiated when the Lyt 5.2 drugcytokerine-treated groups of mice were killed 7 weeks after the sixth cycle of chemotherapy. Femur cells from each group were harvested, washed in Hank's Balanced Salt Solution, and counted in a hemocytometer with viability assessed by trypan blue exclusion. At this time pooled femur cells from each treatment group of mice were serially transferred into 10 recipient Lyt 5.1 mice (1 × 10^7/mouse) that had been lethally irradiated with 1,100 cGy using a 60Co source irradiator. The MRA of the femur cells was determined by the percent survival of these mice 30 days after transfer. The same pools of femur cells were also used to determine Lyt donor versus recipient phenotype and to assay for stem cell CUFS and CUFT activity. Before each bone marrow transfer, donor mice were given sufficient time (8 weeks) for their bone marrows to repopulate. Due to technical difficulties with our irradiator, mice were allowed to recover for 7 months between the first and second serial transfers.

Progenitor cell assays. Pooled bone marrow cell suspensions from each treatment group were counted in a hemocytometer, and viability was assessed by trypan blue exclusion. For the CFU-C colony growth assay, cells were diluted at the appropriate concentration in RPMI-1640 medium (Whittaker M. A. Bioproducts, Walkersville, MD) supplemented with 20% fetal bovine serum (Whittaker M. A. Bioproducts), 1% equine serum (Hyclone Laboratories, Logan, UT), sodium pyruvate, L-serine, 0.6% tryptic soy broth, and 0.3% bacto agar (Difco Laboratories, Detroit, MI). Cells were suspended in flat-bottom 96-well microtiter plates in the assay medium supplemented with 50 U/well GM-CSF, at 5,000, 2,500, 1,000, and 500 cells/well (24 wells/dilution tested), and incubated for 8 to 10 days at 37°C in humidified, and 5% CO2. Wells were scored either positive (> 50 cells in a colony) or negative for colony growth using an inverted microscope at 40× magnification. CFU-C precursor frequency was calculated from the limiting dilution data by the Poisson probability distribution,14,15 using the BMDP-IR software program (BMDP Statistical Software, Los Angeles, CA). CFU-C/femur was calculated by multiplying the mean number of cells/femur by the CFU-C frequency. The Student's t-test was used to determine the equality of the population regression coefficients.

For the determination of progenitor splenic colony formation after each serial transfer, between 2 × 10^5 and 1 × 10^5 harvested bone marrow cells from each treatment group were suspended in phosphate-buffered saline (PBS) and injected intravenously (IV) into lethally irradiated (1,100 cGy) Lyt 5.1 recipient mice (7/group). Macrosopic splenic colonies were counted on day 10 after injection. The Mann-Whitney test was used to determine the significance of the results.

RESULTS

Effect of repeated combined CTX + cytokine therapy on bone marrow hematopoietic capacity. Seven weeks after the sixth cycle of CTX therapy either with or without IL-1
protective or CSF restorative therapy, mice from all treatment groups were killed. Bone marrow cells were then obtained for hematopoietic assays or for adoptive transfer into irradiated recipient mice. The results shown in Table 1 indicate that all mice treated with CTX either with or without cytokine therapy had a reduced femur cell number. Similarly, when the bone marrow cells from these mice were assayed for in vitro CFU-C activity per femur, a reduction was observed in all groups administered CTX therapy, except those that received IL-1 and GM-CSF. However, the reduction in CFU-C activity was correlated with the loss in cell number rather than a significant decrease in the frequency of CFU-Cs in the bone marrow of these mice. The mice receiving IL-1 plus GM-CSF had an unexplainable (though not significantly different from the untreated group of mice) increase in CFU-C frequency.

CFU-S frequency in lethally irradiated hosts was largely unaffected by CTX therapy or CTX therapy combined with any of the cytokine treatments. The only exception was the group receiving IL-1 plus CTX in which CFU-S frequency was significantly reduced by 56%. However, considering the loss of bone marrow cellularity, there would appear to be a reduction in the total CFU-S number in all of the CTX-treated mice, with little or no further reduction in CFU-S activity observed in CTX-plus-cytokine-treated mice. The results obtained from the CTX plus IL-1-treated mice suggest that the IL-1 pretreatment did not prevent a reduction of the CFU-S potential in these mice. However, when $1 \times 10^6$ femur cells from all seven groups of mice were injected separately into groups of lethally irradiated mice, all seven groups of mice demonstrated a 100% survival rate for up to 8 months (data not shown).

Effect of repeated CTX + cytokine therapy on bone marrow hematopoietic capacity as determined by serial transfer of bone marrow cells. Several parameters assessing the hematopoietic function of the groups of mice that received the various CTX cytokine regimens were performed just before the first transfer. The irradiated hosts that received the first bone marrow transfer were allowed to recover and then were killed. Femur cells taken from the Lyt 5.1 recipient mice that received Lyt 5.2 bone marrow cells from either the untreated mice, the CTX-treated mice, or the CTX:IL-1:G-CSF-treated mice 8 months after lethal irradiation and adoptive transfer were all $\geq 65\%$ positive for the Lyt 5.2 leukocyte marker, and $\leq 6\%$ for the Lyt 5.1 marker. It should be noted that in normal mice the Lyt marker is expressed on only 65% to 75% of the femur cells harvested. In general, these data obtained after the first serial transfer suggest that there is no evidence of greater depletion of murine stem cells after the restorative G-CSF or GM-CSF regimens, or by the protective IL-1 regimen versus that observed for the mice administered CTX alone. Hematopoietic parameters were again assessed to determine stem cell function. Cells from these mice were again transferred into other groups of lethally irradiated mice. This process was repeated three times; therefore, the data are presented for each group of mice before the use of these cells for serial transfer.

The ability of serially transferred bone marrow from the treated groups of mice to repopulate the femur of the irradiated recipient mice is shown in Table 2. Through the first and second serial transfers, there does not appear to be any evidence of significant loss of hematopoietic restoration by the original host cells, regardless of the previous CTX or CTX-plus-cytokines treatment. However, by the third bone marrow transfer, mice that received cells from G-CSF- and GM-CSF-treated mice showed a significantly reduced ability to repopulate the femurs (Table 2) compared with the mice in the other treatment groups. Repeated CTX therapy did appear to reduce the restorative capacity of the femur cells by the third transfer, with IL-1 pretreatment having no effect on the CTX-induced reduction in femur cell number. A similar pattern was noted in splenic cellularity (data not shown). Interestingly, treatment with IL-1 before either of the CTX-CSF restorative regimens appeared to prevent the detrimental effects observed when either CSF was administered after CTX therapy.

A more striking pattern emerges when looking at the CFU-C generating capacity of the serially transferred cells, with the pattern emerging only after the second transfer (Table 3). Cells from mice treated with either of the CSFs after CTX administration showed an almost complete disappearance of CFU-C potential. IL-1 administration before CTX and CSF treatment showed a remarkable sparing effect on the CFU-C potential of the bone marrow cells. The CFU-S function of the serially transferred cells,

### Table 1. Effect of Repeated CTX-Cytokine Therapy on Femur Cell Hematopoietic Capacity

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cytokine(s)</th>
<th>Cells/Femur x 10⁶</th>
<th>CFU-C/Femur</th>
<th>CFU-S/Femur x 10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>1.9</td>
<td>4,677*</td>
<td>16 ± 2**</td>
</tr>
<tr>
<td>CTX</td>
<td>None</td>
<td>0.7</td>
<td>2,641</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>CTX</td>
<td>IL-1</td>
<td>1.2</td>
<td>3,433</td>
<td>7 ± 2**</td>
</tr>
<tr>
<td>CTX</td>
<td>G-CSF</td>
<td>1.1</td>
<td>1,635</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>CTX</td>
<td>GM-CSF</td>
<td>1.1</td>
<td>1,880</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>CTX</td>
<td>IL-1 + G-CSF</td>
<td>1.0</td>
<td>1,840</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>CTX</td>
<td>IL-1 + GM-CSF</td>
<td>1.4</td>
<td>8,891</td>
<td>19 ± 2</td>
</tr>
</tbody>
</table>

*CT7BL/6 Lyt 5.2 mice were administered six cycles of drug therapy either with or without IL-1 pretreatment or growth factor posttreatment. Seven weeks after administration of the last cycle of therapy, femur cells were obtained from all groups of mice and assayed for CFU-C and CFU-S activity.

*No significant differences when compared with all other groups.

†No significant differences when compared with all other groups except the CTX + IL-1 group ($P < .03$).

‡Significant ($P < .003$) when compared with all groups except the CTX + G-CSF group ($P < .07$).
treated Lyt 5.2 mice were serially transferred by IV injection into Lyt 5.1 mice. For each group, the number of mice killed from each group was even further reduced by the third transfer, with both groups showing a dramatic reduction (P < .0001) when compared with control group. IL-1 pretreatment, given before CTX, significantly (P < .05) increased the CFU-S capacity of mice administered CTX therapy. Mice pre-treated with IL-1 before the CTX-plus-G-CSF combination showed a significantly higher ability (P < .0001) to generate CFU-S colonies than mice administered CTX plus G-CSF alone. Although CFU-C capacity was significantly spared in GM-CSF mice pretreated with IL-1, a similar, but less substantial improvement of CFU-S capacity (P < .02) was observed when the CTX-plus-GM-CSF mice were pretreated with IL-1. Together the spleen and marrow reconstitution data demonstrate that restorative therapy with either G- or GM-CSF may significantly reduce the long-term stem cell function of CTX-treated mice.

Table 2. Effect of Repeated CTX-Cytokine Therapy on Serially Transplanted Femur Cells to Repopulate the Femurs of Lethally Irradiated Host Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cytokine(s)</th>
<th>Cells/Femur ( \times 10^7 ) Obtained Before the (nth) Serial Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>1.9, 1.4, 1.9</td>
</tr>
<tr>
<td>CTX</td>
<td>None</td>
<td>0.7, 1.6, 1.2</td>
</tr>
<tr>
<td>CTX</td>
<td>IL-1</td>
<td>1.2, 1.8, 1.1</td>
</tr>
<tr>
<td>CTX</td>
<td>G-CSF</td>
<td>1.1, 1.6, 0.7*</td>
</tr>
<tr>
<td>CTX</td>
<td>GM-CSF</td>
<td>1.1, 1.3, 0.7*</td>
</tr>
<tr>
<td>CTX+</td>
<td>IL-1 + GM-CSF</td>
<td>1.0, 1.9, 1.8</td>
</tr>
<tr>
<td>CTX+</td>
<td>IL-1 + GM-CSF</td>
<td>1.4, 2.1, 1.7</td>
</tr>
</tbody>
</table>

1 \( \times 10^7 \) pooled femur cells (7 to 10 mice/group) obtained from treated Lyt 5.2 mice were serially transferred by IV injection into Lyt 5.1 mice irradiated with 1,100 cGy. Before each serial transfer, each group was measured for the number of pooled femur cells obtained for each group by the number of mice killed from each group. Thus, the numbers represent mean values per femur.

*The mean number of cells/femur from these two groups were significantly lower (P = .029 by t-test) than the mean number of cells/femur from the other five groups of animals.

Table 4. Effect of Repeated CTX-Cytokine Therapy on Serially Transplanted Femur Cells’ Ability to Generate CFU-S

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cytokine(s)</th>
<th>CFU-S Generated by Cells Obtained Before the (nth) Serial Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>19 ± 2, 152 ± 27, 22 ± 25</td>
</tr>
<tr>
<td>CTX</td>
<td>None</td>
<td>16 ± 1, 130 ± 21, 15 ± 2</td>
</tr>
<tr>
<td>CTX</td>
<td>IL-1</td>
<td>7 ± 2, 53 ± 23, 25 ± 2</td>
</tr>
<tr>
<td>CTX</td>
<td>GM-CSF</td>
<td>12 ± 3, 30 ± 1, 10 ± 2</td>
</tr>
<tr>
<td>CTX</td>
<td>G-CSF</td>
<td>24 ± 2, 35 ± 3, 9 ± 3</td>
</tr>
<tr>
<td>CTX+</td>
<td>IL-1 + G-CSF</td>
<td>17 ± 1, 69 ± 14, 25 ± 2</td>
</tr>
<tr>
<td>CTX+</td>
<td>IL-1 + GM-CSF</td>
<td>19 ± 2, 45 ± 15, 15 ± 41</td>
</tr>
</tbody>
</table>

1 \( \times 10^7 \) pooled femur cells (7 to 10 mice/group) obtained from treated Lyt 5.2 mice were serially transferred by IV injection into Lyt 5.1 mice irradiated with 1,100 cGy. Before each serial transfer, each group was measured for the number of pooled femur cells obtained for each group by the number of mice killed from each group. Thus, the numbers represent mean values per femur.

*No significant differences among any groups.

†Significant (P < .05) when compared with all other groups.

therapy also showed a significantly higher ability (P < .0001) to generate CFU-S colonies than mice administered CTX plus G-CSF alone. Although CFU-C capacity was significantly spared in GM-CSF mice pretreated with IL-1, a similar, but less substantial improvement of CFU-S capacity (P < .02) was observed when the CTX-plus-GM-CSF mice were pretreated with IL-1. Together the spleen and marrow reconstitution data demonstrate that restorative therapy with either G- or GM-CSF may significantly reduce the long-term stem cell function of CTX-treated mice.

Determination of MRA by survival and donor cell phenotype after bone marrow serial transfers. Serially transferred bone marrow cells from various treatment groups were given the functional test for marrow repopulating ability. As shown in Table 5, these cells were assayed for their ability to permit survival 30 days after the transfer of 1 \( \times 10^7 \) cells into mice treated with a lethal dose of irradiation (1,100 cGy). As with the CFU-C and CFU-S data, cells from the CTX plus G-CSF- and GM-CSF-treated mice showed a markedly reduced (30% and 57% survival, respectively) MRA ability with the third serial transfer. IL-1 prevented these negative effects because both of the CSF groups showed a 90% to 100% survival rate if they were treated with IL-1 before CTX. The Lyt phenotype of the femur cells in the recipient mice of all groups remained predominantly of the Lyt 5.2 donor phenotype. However, after the third and final serial transfer when the stem cell function started to decrease and the MRA was reduced for the CSF-treated groups, the percentage of marrow cells expressing the Lyt 5.2 phenotype of the donor cells derived from the CSF-treated mice decreased markedly, as shown in Table 6. The G-CSF- and GM-CSF-treated groups showed...
Hematopoietic stem cells have been defined as cells capable of prolonged self renewal and which can proliferate and differentiate into several cell types. It has been well established by serial transplantation studies\(^{16-18}\) that stem cells have a defined capacity to proliferate and renew, although marrow failure is rarely of clinical concern because the hematopoietic proliferative capacity usually far exceeds the mammalian life span. However, current clinical trials that incorporate repeated cycles of myelotoxic chemotherapy with concurrent repeated myelostimulatory growth factor therapy pose new concerns about the potential for hematopoietic damage and a greater risk of developing marrow failure.

The CTX:C57B1/6 model was selected for this study for several reasons. The alkylating agent cyclophosphamide is considered less toxic to the self-renewal capacity of marrow than radiation therapy or other alkylating agents, although the acute damage to the hematopoietic system may appear equally as severe.\(^{13}\) Second, the C57B1/6 mouse is a long-lived strain\(^{19}\) with a low fraction of dividing stem cells.\(^{20}\) Therefore, the stem cell component of the bone marrow cells in this strain may function through many stages of serial transplantation, allowing a broad time span over which decreased stem cell activity may develop.

Hematopoietic assays performed 7 weeks after the last of the six drug:cytokine cycles yielded results similar to those observed in past studies.\(^{21}\) We noted that the hematopoietic potential of stem cells from mice treated with the six cycles of CTX may have been reduced, as suggested by both a reduction of the femur cell number and a loss (though not significant) of CFU-C activity in these mice (Table 1). However, there was no loss of CFU-S activity observed at this point (Table 4), a finding consistent with previous observations by others that CTX spares the more immature hematopoietic stem cells.\(^{21}\) Furthermore, the data presented in Table 1 do not suggest that the IL-1 pretreatment or G- or GM-CSF restorative regimens, either alone or in combination with CTX, further reduced the hematopoietic potential over that already observed in mice administered the CTX regimen alone. This result suggests that cytokine-chemotherapeutic regimens offer little potential for hematopoietic damage.

However, if we examine these same parameters through the series of bone marrow (femur cell) transfers into irradiated hosts, compromised stem cell function can be detected. A decrease in cell number was seen after the third serial transfer of cells derived from the CTX-treated mice that had received restorative G- or GM-CSF. Interestingly, recipient mice that had received cells from the IL-1 pretreatment plus G- or GM-CSF posttreatment groups did not show this trend in the femur (Table 2).

Cells from CTX-plus-CSF--treated mice indeed showed a much greater loss of CFU-C and CFU-S activity when compared with the loss observed for cells from untreated...
mice or CTX-treated mice. Because CTX has been found to be more sparing of early hematopoietic stem cells, it is not surprising to note that the decrease of CFU-C activity, representing a more mature precursor stem cell, was observed in the CTX-treated mice before the cells were placed into serial transfer. In contrast, the CFU-S activity of femur cells from CTX-treated mice, representing an earlier precursor cell, did not show obvious signs of decreased activity until after the serial transfer phase had begun. However, both the G-CSF and GM-CSF treatment regimens precipitated a much greater decrease in both the CFU-S and CFU-C compartment. These results suggest that the myelostimulatory CSF regimens may be placing normally CTX resistant stem cells in a state more sensitive to CTX toxicity.

Not surprisingly, and most convincingly, the reduced MRA in cells derived from growth factor-plus-CTX-treated mice, shown in Table 5, correlated very well with the other hematopoietic parameters, i.e., femur CFU-C, and CFU-S colony production. Mice pretreated with IL-1 showed very little difference in activity when compared with cells from mice administered CTX cycles without cytokines. Furthermore, IL-1 pretreatment reduced the detrimental effects of the CSF restorative regimens. This trend was observed in every hematopoietic assay examined as significant differences were observed in the study for IL-1 and G-CSF–treated mice, as well as a suggestive reduction in femur repopulating ability. IL-1 may act to expand the stem cell compartment and it may induce cytokines such as tumor necrosis factor and interferon-γ, which may inhibit stem cell expansion. It is possible that the inhibitory effects of IL-1–induced cytokines may protect the stem cell from CTX-induced damage while the CSFs recruit stem cells into a more CTX-susceptible state. It is not clear what effect of IL-1 may be dominant in vivo. These results may also be explained by the prolongation of survival of both GM-CFU and burst-forming units-erythroid (BUFE) hematopoietic progenitors by IL-1.

In past serial transplantation studies of stem cell renewal capacity, conflicting results have been reported concerning the eventual reduction in self renewal and CFU-S activity, or a lack of such a reduction on serial transfer. These differing interpretations may have resulted from undetected autologous bone marrow reconstitution occurring through the serial transfer process. To reduce the likelihood that autologous reconstitution was influencing the outcome of this study, all femur cell preparations harvested after each serial transfer were tested to determine if the predominant phenotype remained that of the original Lyt 5.2 donor. By irradiating the recipient mice with 1,100 cGy and transferring 1 × 10⁷ cells, it became apparent that the donor cell Lyt 5.2 phenotype was the predominant source of cells repopulating the recipient mice throughout the study in all the groups tested up to the last of the serial transfers. Only at the last transfer, as shown in Table 6, did the CTX-plus-CSF–treated mice show a dramatic inability of the Lyt 5.2 donor cells to repopulate the irradiated recipient mice. As with the other assays, the IL-1-plus-CTX plus-CSF–treated mice did not show this loss of MRA by Lyt 5.2 donor cells. Failure of transferred bone marrow to reconstitute was also associated with a larger degree of autologous bone marrow reconstitution by the irradiated Lyt 5.1 host mice, suggesting that the long-term Lyt 5.2 marrow repopulating stem cell had been more rapidly depleted by the involvement of the CSF regimens but the short-term stem cell activity allowed some mice to survive the early radiation-induced myelosuppression. Because the Lyt 5.2 cells were no longer capable of maintaining the recipient’s hematopoietic system in the long term, the damaged Lyt 5.1 recipient marrow partially restored some marginal marrow function. Thus, a high proportion of the mice failed to survive the third serial transfer, even though a very high inoculum of bone marrow cells was injected into the recipient mice.

Unlike most preclinical studies in which the effects of a single dose of chemotherapy was evaluated, this investigation with repeated cycles of therapy was designed to mimic the therapeutic regimen that might be more clinically applicable toward the treatment of human cancers. In conclusion, we have found that the repeated use of either G-CSF or GM-CSF as a restorative regimen after cyclic CTX therapy may seriously reduce the MRA or stem cell capacity. In contrast, IL-1 pretreatment significantly reduced many of the harmful effects of the CSF restorative regimens on the host stem cell capacity.

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Hematopoietic stem cell depletion by restorative growth factor regimens during repeated high-dose cyclophosphamide therapy [see comments]

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