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J Immunol 2003; 171:1202-1206; doi: 10.4049/jimmunol.171.3.1202
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Hemopoietic Function After Use of IL-1 with Chemotherapy or Irradiation

Renee V. Gardner,2* Evangeline McKinnon,* Connie Poretta, † and Lily Leiva‡

IL-1 has putative chemo- and radioprotective properties, but its effects on primitive hemopoietic stem cell (PHSC) and early multilineage precursor function when given with these modalities is unknown. C57BL/6/J (B6) mice, given IL-1 20 h before cyclophosphamide (200 mg/kg for four biweekly doses) or before irradiation (500 cGy), were sacrificed after 4 wk. Their marrow was used as donor cells, and that from B6-Hbb<sup>Gpi1a</sup> (B6-GPI) mice was used as competitor cells in competitive repopulation. Percentages of B6 cells were measured at 30 and 150 days. Stem cell numbers were estimated using binomial statistics. IL-1 alone did not affect stem cell function. As expected, significant declines in early multilineage precursor and PHSC function occurred with chemotherapy and radiation alone. IL-1 with chemotherapy led to exacerbation of these losses in function and numbers (p < 0.05). A similar reduction in function occurred using IL-1 before irradiation. In summary, IL-1 with chemotherapy or radiation worsened chemotherapy- and radiation-induced functional damage to PHSC and other hemopoietic precursors, suggesting that improvements in survival do not necessarily translate into preservation of hemopoietic function. The Journal of Immunology, 2003, 171: 1202–1206.

Both chemotherapy and radiation cause severe, permanent impairment of hemopoietic function (1–6). In the experimental model, postradiation survival has been shown to be influenced by the extent and rate of recovery of committed progenitors such as the CFU-Spleen (CFU-S)3 (7). Since cytokines stimulate the recovery of CFU-S and other committed hemopoietic precursors after both treatment modalities, they have been proposed as chemo- and radioprotectants (8–18). Prominent among the cytokines investigated for their protective properties is IL-1. A single dose of IL-1 injected 20 h before irradiation led to a 75–90% survival rate in mice after an otherwise lethal dose of irradiation (13). This favorable outcome was believed to be due to transient enhancement of endogenous hemopoiesis after IL-1, since excellent survival rates were observed regardless of the relatively low doses of exogenous marrow cells administered for engraftment (12). Impressive increases in splenic and marrow cellularity and CFU-S number were observed after IL-1, and it was proposed that marrow transplantation after irradiation could actually be supplemented partially by IL-1 (14).

Improvements in survival after chemotherapy are not quite as dramatic as those seen after irradiation. However, the use of IL-1 as a single or multiply administered agent in one study resulted in a survival rate of 40–80% when IL-1 was given 24 h before a sublethal dose of cyclophosphamide (13). Variable, but promising, protection was noted with other anticancer drugs as well (18–20). The improved survival after chemotherapy similarly appeared to be due to acceleration of myeloid recovery (17). Most studies have focused on the recovery of peripheral blood cells, in vitro colony-forming ability, or in vivo CFU-S (21) numbers (in mice) (8–18). These parameters only allow analysis of the function of already differentiated or committed cell populations. The tests cannot predict the behavior or number of the most primitive hemopoietic stem cells (PHSC) after treatment; for instance, it has been demonstrated that estimations of PHSC numbers based on CFU-S concentrations are inaccurate, being 10–100 times higher than their actual values (22–24).

Little is known about the effects of IL-1 on PHSC. These cells are pluripotential progenitors and account for life-long marrow reconstitution, comprising not >0.001–0.05% of total bone marrow (25, 26). The existing data are conflicting (27, 28). In one study, IL-1 reversed the exacerbation of chemotherapy-induced damage to PHSC observed after G-CSF was given with cyclophosphamide (27). IL-1 by itself offered little protection. In another study, the preadministration of IL-1 with cyclophosphamide led to a significant decline in PHSC function and marrow progenitor numbers (28). We have therefore studied PHSC function and stem cell numbers using the model of competitive repopulation (29).

Materials and Methods

Mice

Mice used in these experiments were obtained from The Jackson Laboratory (Bar Harbor, ME) and were maintained in pathogen-free conditions in an American Association for Laboratory Animal Care-accredited facility. C57Bl/6 (B6) mice were used as donors for competitive repopulation, while the congenic strain, C57Bl6/Hbb<sup>Gpi1a</sup> (B6-GPI), was used as competitors. These strains have identical genetic background, differing only in their hemoglobin and glucose phosphate isomerase loci. Their cells are identified by differences in electrophoretic migratory characteristics, making their identification on electrophoresis and their quantitation using densitometry simple to achieve. For instance, B6 mice are Hbb<sup>Gpi1a</sup>. They have a single (s) hemoglobin allele that forms an electrophoretic band that migrates rapidly from negative to positive on standard cellulose acetate gels. The Gpi<sup>1a</sup> allele forms a glucose phosphate isotype that migrates more rapidly than the Gpi<sup>1b</sup> isotype. B6-GPI mice have cells that bear diffuse (d) hemoglobin with two distinct bands with intermediate and slow...
migratory patterns on electrophoresis. Their GPI isotype, GPI\textsuperscript{a}, migrates more slowly than that of B6 mice.

Chemotherapy schedule and competitive repopulation assay

A schematic of the planned experiments is shown in Fig. 1A. Groups of B6 mice, 8–12 wk old, were given cyclophosphamide (Sigma-Aldrich, St. Louis, MO) only at a dose of 200 mg/kg i.v. or after a single i.p. dose of 1 μg IL-1 (R&D Systems, Minneapolis, MN), at 1 h before irradiation with 300 cGy. Mice were sacrificed 4 wk after the last treatment, and the marrow was then placed in a single-cell suspension using DMEM (Sigma-Aldrich). Donor (B6) and competitor (B6-GPI) cells were mixed together with a final concentration of 10^6 cells of each type. Equal aliquots of this mixture were injected into lethally irradiated (1100 rad) B6 mice of the same age and sex. Recipients were bled by retro-orbital puncture at 30 and 150 days. Erythrocytes and lymphocytes were separated by density gradient centrifugation. The cells so separated were then prepared for electrophoresis, and the amount of donor-type hemoglobin or glucose phosphate isomerase was measured by densitometric analysis of the gels. An example of such a gel analysis is shown in Fig. 1B.

Statistical analysis

The calculation of repopulating units (RU) using a binomial statistical model (25) allows assessment of the number of those cells among the donor cell population that are capable of marrow reconstitution (25). This model works on the assumption that the contribution of lymphoid and erythroid populations is the same for every PHSC, whether donor or competitor. RU refers to all cells, not just the most primitive ones, that are responsible for reconstitution of the marrow after myeloablation. It gives an accurate assessment of functional capacity. One RU is defined as the repopulating ability of 10^6 untreated competitor cells. If the donor population functions normally, 10^6 cells should contain ~1 RU and repopulate as well as a comparable number of untreated competitor marrow cells. On the other hand, if fewer RU are present, this finding suggests that the available cells among the donor cell population that are capable of marrow reconstitution are reduced in number, that the repopulating ability per cell is reduced, or both. RU are calculated using the following formula: RU = (%)(number of 10^6 competitor cells used)/(100% - %), where % is percentage of donor-type cells (25).

The results of competitive repopulation and other data were collated, and the means were calculated. Results are shown as the means ± SEM. Significance was determined using the INSTAT statistical program (GraphPad, San Diego, CA) for performance of variance analysis. Significance was determined using the Student-Newman-Keuls t test, with p ≤ 0.05 indicating statistical significance.

Results

Effects of IL-1 with irradiation

We assessed function of both short term and exhaustible multilineage precursors (EMP) at 30 days and that of PHSC at 150 days (25). EMP lack the long term repopulating capacity of PHSC and are exhaustible; however, they repopulate both lymphoid and myeloid systems, unlike more differentiated precursors that are identified through use of the spleen colony-forming assay (30–33).

The results of electrophoresis at both early and late time points are shown in Table I. The function of EMP and PHSC was severely impaired after sublethal irradiation. EMP function declined to slightly <30–40% of their normal levels; the control B6 percentage was 53 ± 2%, and that of the radiation only group was 21 ± 6%. IL-1 alone had very little impact on EMP repopulating ability. A drop in EMP repopulating ability to 17 ± 4% was noted, however, when IL-1 was combined with sublethal irradiation.

IL-1 brought about an increase in PHSC marrow repopulating ability from 50 ± 3 to 66 ± 4%, which was not due to an increase in total marrow cell numbers. With irradiation, repopulating ability among PHSC decreased to 14 ± 5%. Combining irradiation with IL-1 led to a further reduction of repopulating ability to 6 ± 2%. For both EMP and PHSC, the differences between irradiation and control groups were significant (p < 0.001). RU concentrations
Results of competitive repopulation experiments after IL-1 and sublethal irradiation at 30 days

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>BM cell no.</th>
<th>% Donor Cells</th>
<th>RU Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.4</td>
<td>53 ± 2</td>
<td>11</td>
</tr>
<tr>
<td>XRT</td>
<td>5.2</td>
<td>21 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-1</td>
<td>5.4</td>
<td>47 ± 2</td>
<td>9</td>
</tr>
<tr>
<td>IL-1/XRT</td>
<td>4.9</td>
<td>17 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results of competitive repopulation experiments are tabulated as the mean ± SEM. Experiments were repeated three times and mice were bled at 30 days to obtain the percentage of donor (B6) cells and determine EMP. BM, bone marrow cells, with numbers given ×10<sup>5</sup> cells: XRT, sublethal irradiation administered at a dose of 500 cGy. IL-1 was given at a dose of 1 μg i.p. either alone or 20 h before the irradiation dose was administered (IL-1/XRT). Controls received no treatments. The RU concentration is per 10<sup>6</sup> competitor cells, calculated using the formula indicated in Materials and Methods.

<sup>b</sup> Significant differences between the treatment group and controls (p < 0.001). While RU are not shown here as the mean ± SEM, analysis was performed using these values to determine statistical significance of differences between these treatment groups and controls. Such differences are indicated (31).

Effects of IL-1 therapy with chemotherapy

In experiments using a combination of IL-1 and the chemotherapeutic agent cyclophosphamide (Table II), EMP function was slightly less than expected for controls (44 ± 2%), although marrow cell numbers were not significantly lower than we have previously seen. For that reason the repopulating ability of EMP after cyclophosphamide use was not significantly different from that of controls. However, using IL-1 with cyclophosphamide caused a significant reduction in marrow repopulating ability from 37% after cyclophosphamide alone to 28% (p < 0.01). The RU concentration for these short term precursors was reduced from control levels (7.9/10<sup>6</sup> cells) for treatment groups receiving cyclophosphamide (to 5.9/10<sup>6</sup> cells for cyclophosphamide alone and to 3.9/10<sup>6</sup> cells for combination therapy). Animals receiving IL-1 alone incurred no losses.

As previously indicated, IL-1 alone had no deleterious effect on PHSC marrow repopulating ability. Cyclophosphamide use led to a drop in donor marrow percentage from 49 ± 3 to 32 ± 2% for controls (p < 0.001). There was a further significant decrease in the donor marrow repopulating ability to 22 ± 3% after IL-1 was administered before cyclophosphamide (p < 0.05).

With administration of cyclophosphamide alone, the RU concentration was halved; concentration was recorded as 5/10<sup>6</sup> competitor cell numbers were not significantly lower than we have previously seen.

Results of competitive repopulation experiments after IL-1 with irradiation at 150 days

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>% Donor Cells</th>
<th>RU Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50 ± 3</td>
<td>10</td>
</tr>
<tr>
<td>XRT</td>
<td>14 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-1</td>
<td>66 ± 4</td>
<td>19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-1/XRT</td>
<td>6 ± 2</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;,c</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results are shown of competitive repopulation at 150 days. These results are representative of PHSC function, with donor or B6 marrow percentage displayed as the means ± SEM. Abbreviations are given in Table I, n, Number of animals in each treatment group.

<sup>b</sup> Statistical significance of differences between treatment groups (p < 0.001).

<sup>c</sup> Differences that are significant between irradiation only and combined therapy groups (p < 0.01).

Discussion

IL-1 and other cytokines are reputed to have a beneficial role after chemotherapy or irradiation. This assessment has stemmed from better survival rates with their use (13) or improved cell recovery and colony-forming ability (13, 34). The improved survival after IL-1 with chemotherapy or irradiation is thought to be largely the result of its ability to induce hematologic recovery (7, 12), a hypothesised by observations that IL-1 is most effective in protecting against myelotoxic drugs rather than those that are primarily nephrotoxic or cardiotoxic (18).

Survival is usually measured at ~30 days, a relatively short period of time from treatments. This period does not allow adequate time for measurement of the deleterious effects of treatments on hemopoiesis, something that may not become apparent until months later. Therefore, survival and hematologic function can have disparate measurements. This observation is supported by the reports of several investigators. For example, in experiments performed by Botnick et al. (35) mice survived radiation and chemotherapy with severe and limiting hemopoietic (or other organ system) damage that was not at first apparent. Animals appeared well, living for prolonged periods of time, but they suffered profound depletion of reproductive, connective tissue, and hemopoietic stem cells (35). In experiments performed by Futami et al. (36) it was noted that mice could be successfully protected by IL-1 from the acute hematologic toxicities of cyclophosphamide, but could still experience late deaths because of progressive and fatal damage to petittor cells, as opposed to the control group, where RU concentration was 10/10<sup>6</sup> cells. Addition of IL-1 to the treatment regimen resulted in a further drop in RU concentration and numbers. Although RU results for both treatment groups were significantly different from the control values, the differences between IL-1 with cyclophosphamide and cyclophosphamide only groups was itself insignificant. Total RU numbers paralleled these results.

Results of competitive repopulation after IL-1 and cyclophosphamide

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>No. of Marrow Cells (×10&lt;sup&gt;6&lt;/sup&gt;)</th>
<th>RU Conc. (×10&lt;sup&gt;6&lt;/sup&gt;)</th>
<th>Total RU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44 ± 2</td>
<td>5.4</td>
<td>427</td>
</tr>
<tr>
<td>CTX</td>
<td>37 ± 2</td>
<td>5.9</td>
<td>348</td>
</tr>
<tr>
<td>IL-1/CTX</td>
<td>28 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.4</td>
<td>250</td>
</tr>
<tr>
<td>IL-1 only</td>
<td>47 ± 2</td>
<td>5.4</td>
<td>481</td>
</tr>
</tbody>
</table>

<sup>a</sup> Experiments were performed in triplicate. The percentage of donor or B6 marrow is shown as the means ± SEM. IL-1 was given at a dose of 1 μg i.p. either alone or 20 h before irradiation. CTX, cyclophosphamide at a dose of 200 mg/kg i.v. bi-weekly for five doses. The table shows the results of 30-day assessments of EMP repopulating ability, n, number of animals in each treatment group.

<sup>b</sup> Differences from control that are significant (p < 0.01). Significant difference between cyclophosphamide only and combined therapy use (p < 0.05).
organs other than the hemopoietic system. However, more prolonged observation times than those usually used for IL-1/chemo- or radiotherapy experiments were required to see this effect. Our results underscore then the fact that survival can improve regardless of damage to vital organs.

We have thus concentrated on examining the effects of IL-1 on hemopoietic precursors, EMP at 30 days and PHSC at 150 days after treatments. As noted, IL-1 alone had no negative impact on EMP or PHSC, but significant reductions in both EMP and PHSC function occurred after the use of IL-1 with either irradiation or cyclophosphamide. Stem cell numbers, whether EMP or PHSC, were also significantly adversely affected, dropping by as much as 67%. These defects remained 5–6 mo after treatment. They are consistent with the IL-1-induced hemopoietic defect observed in previous experiments reported by us, testing the effect of GM-CSF on hemopoiesis (28). Then, IL-1 given before cyclophosphamide with GM-CSF resulted in exacerbation of hemopoietic deficits already present after either cyclophosphamide or cyclophosphamide and GM-CSF (28).

To our knowledge, only one other group has performed studies looking at the effect of IL-1 on PHSC. In the hands of Hornung and Longo (27), IL-1 had no deleterious effect on hemopoiesis when used alone, but was restorative of hemopoietic function when given before a combination of cyclophosphamide and GM-CSF. Their study involved the use of serial transplantation (37) to ex-
amine the effects of treatment on hemopoietic progenitors. We have used instead the competitive repopulation assay (25). We think that our use of this assay may in part be responsible for data that appear to be at variance with those of the other investigators, since it allows a more sensitive dissection of the diverse contribu-
tions of PHSC and other early stem cell populations to marrow repopulating effort, permitting both quantitative and qualitative analyses of PHSC function that are not possible with serial trans-
plantation (25, 38).

There are a few properties of IL-1 that might account for these excessive PHSC losses. For one, IL-1, an inflammatory cytokine, may induce the production of reactive oxygen intermediates in excessive quantities (39). If so, cellular, protein and nucleic acid damage through lipid peroxidation could occur with ensuing dam-
age to hemopoietic precursors.

Neta et al. (40) hypothesized that IL-1, by spurring forward hemopoietic stem cell cycling, protected cells from radiation dam-
age; on the other hand, it brought about protection against dele-
tious chemotherapy effect by inhibiting cells from cycling before chemotherapeutic injury (41). However, we and others have pre-
viously presented evidence that suggests that chemotherapy, in-
ducing more rapid entry into S phase, makes hemopoietic cells more vulnerable to the injury caused by cytotoxic agents (42–48). Hemopoietic stem cells, now in an accelerated cycling phase, may experience excessive proliferative stress. This, in turn, may lead to eventual stem cell exhaustion in both number and function (42).

Normally, the proliferative capacity of PHSC would greatly ex-
ceed the replicative requirements of the animal’s life span (49). However, Mauch and associates (43) demonstrated that a perma-
nent loss in stem cell self-renewal capacity occurred after a small number of stem cells, in tibias shielded from otherwise total body irradiation, were required to support hemopoiesis for the entire animal after administration of lethal irradiation doses.

In another study greater sensitization of stem cell compartments, especially of rapidly turning over tissues such as lip mucosa, was observed when IL-1 was given in conjunction with chemotherapy (20). Another example of the possible role of accelerated stem cell cycling is seen after the use of supposedly stem cell-sparing drugs (44, 45). Low doses of cyclophosphamide or 5-fluorouracil are known to spare hemopoietic stem cells when given as a one-time dose. However, these doses cause irreparable damage to PHSC when given just several days apart, at a time when cells have entered the S phase, again suggesting a relationship between replicative defect and decrease in cell cycling (44, 45). Similarly, other cytokines that induce cell cycling, e.g., stem cell factor, have also been noted to be deleterious to the hemopoietic repopulating effort when given before irradiation or chemotherapy (47, 48) despite an increase in progenitor cell number and improved L50 among mice.

Both chemotherapeutic agents, such as cyclophosphamide, and irradiation function by inducing DNA damage, such as double-strand breakage or intercalation (50). Such damage is, at times, reparable if the cell has an adequate repair system or there is sufficient time for repair to take place (46). It is possible that the administration of IL-1, by decreasing cell cycle transit time, may shorten the window of opportunity needed by the cell to repair DNA damage completely and inerrantly (50).

Alternatively, IL-1, with and without either chemotherapy or irradiation, could induce the production of additional cytokines, such as GM- or G-CSF, that have been shown to adversely affect hemopoietic effort (46). It has also been observed that irradiation can alter the marrow microenvironment, possibly inducing high amounts of growth factors, or affect its ability to support either homing or hemopoiesis (46). IL-1, in fact, is also capable of in-
ducing adhesion molecules, such as VCAM-1 or ICAM-1 (46). Imbalance of microenvironmental factors or cytokines could conceivably prevent adequate hemopoiesis from taking place.

Our results demonstrate yet again that cytokines used with anti-
cancer therapy may not always have the beneficial effect in-
tended, since they are given well in excess of physiologically nor-
mal amounts. Assiduous examination of the full impact of their use is necessary to evaluate long term implications.

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