Interleukin-1 Administration Before Lethal Irradiation and Allogeneic Bone Marrow Transplantation: Early Transient Increase of Peripheral Granulocytes and Successful Engraftment With Accelerated Leukocyte, Erythrocyte, and Platelet Recovery

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Administration of interleukin-1 beta (IL-1β) before a lethal irradiation with or without allogeneic bone marrow transplantation (BMT) protects greater than 90% of the irradiated mice. To approach the mechanisms responsible for the radioprotective effect of IL-1, we examined the effects of IL-1 pretreatment on engraftment and kinetics of peripheral blood, spleen, and marrow cell reconstitution after irradiation and BMT. Although the BMT was not necessary for the survival of the IL-1--pretreated lethally irradiated mice, allogeneic marrow did engraft in these mice as evaluated in the spleen and marrow 2 months after BMT. IL-1 pretreatment significantly accelerated hematopoietic recovery versus transplanted saline-treated controls with a pronounced enhancement of peripheral leukocyte, platelet, and erythrocyte recovery. Leukocyte recovery in IL-1--pretreated mice was unique in that IL-1 first induced an early transient (maximum at day 7) increase of peripheral granulocytes before accelerating leukocyte recovery after day 11. IL-1 pretreatment also significantly enhanced marrow cell recovery after allogeneic BMT with an eightfold increase in marrow cellularity from day 4 to 11 versus control transplanted mice. When lethal irradiation was not followed by allogeneic BMT, IL-1 pretreatment also affected the peripheral reconstitution of leukocytes, platelets, and erythrocytes. Interestingly, in the absence of BMT, IL-1 also induced an early circulation of peripheral granulocytes. Overall, our data demonstrate that a single administration of IL-1 before lethal irradiation and allogeneic BMT can induce an early transient increase of circulating granulocytes, followed by an accelerated multilineage recovery and long-term allogeneic engraftment.

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INTERLEUKIN-1 (IL-1) possesses a wide spectrum of inflammatory, metabolic, physiologic, hematopoietic, and immunologic activities.1,2 In vivo, one of the most dramatic effects of this cytokine is the capacity of a single administration of IL-1 to protect mice from an otherwise lethal irradiation-induced and/or alkylating agent-induced myelosuppression.3,4 The exact mechanism responsible for this protective effect of IL-1 is still unknown. An accelerated hematopoietic recovery,5,6 as well as an effective radioprotection7 of hematopoietic progenitors, are two nonexclusive mechanisms through which IL-1 could be operative. When administered before lethal irradiation and allogeneic T-cell–depleted bone marrow transplantation (BMT), IL-1, as expected, confers a considerable survival advantage.8 More surprising is the fact that, although the marrow transplant is not necessary for survival, IL-1 pretreatment does not appear to interfere with allogeneic engraftment as measured in the spleen 2 months after BMT,8 thus highlighting the possible usefulness of such an approach in clinical BMT. However, the effects of IL-1 pretreatment on clinically relevant endpoints such as peripheral blood cell reconstitution or long-term marrow chimerism after allogeneic BMT have not been investigated.

Therefore, we designed experiments to (1) explore the engraftment issue in IL-1–pretreated allogeneic BM recipients and (2) examine the influence of IL-1 pretreatment on peripheral blood, spleen, and marrow hematopoietic recovery after BMT. Because of the profound effects of IL-1 treatment on survival after lethal irradiation independently of transplantation, we also examined hematopoietic recovery after IL-1 treatment and irradiation alone.

MATERIALS AND METHODS

Mice. Male, 8- to 12-week-old BALB/c (H-2d) and CB6F1 (H-2db) were used. Mice were purchased from IFFA-CREDO (L’Arbresle, France).

IL-1 treatment. Recombinant human IL-1β was kindly provided by Dr N. Vita of SANOFI (Labege, France). The preparation was used on a weight basis and had a specific activity of 1.01 × 1088 U/mg by the thyomocyte comitogen assay. IL-1 was diluted to the desired concentration in phosphate-buffered saline (PBS) just before intraperitoneal (IP) injection in a volume of 0.2 mL 20 hours before irradiation. Control mice received 0.2 mL of PBS IP.

Total body irradiation. Groups of 8 to 10 BALB/c mice were placed in a Plexiglas container and given a single dose of total body irradiation of 700 to 800 cGy at 80 cGy/min using a cobalt source (GAMMATRON 3; Siemens, Paris, France) (Radiotherapy Department, Jean Minjoz Hospital, Besançon Plastique, France). Previous studies determined that 700 to 800 cGy was a lethal dose for BALB/c mice within 8 to 16 days, which is the usual course for hematopoietic toxicity (Fig 1A).

Bone marrow transplantation. A semiallogeneic F1 into parent BMT model was chosen to eliminate graft-versus-host disease (GVHD) while preserving postirradiation host-antidonor reactivity. Pooled BM cell suspensions were prepared in RPMI 1640 (Flow Labs, Irvine, CA) from the femurs of CB6F1 mice. The cell suspensions were washed, and 1 × 10E5 BM cells were adjusted in 0.2 mL of PBS for intravenous (IV) administration 12 hours after irradiation. Groups of 8 to 10 IL-1–treated or saline-treated transplanted mice were housed in an animal room with limited access conditions and given sterile fluids and food ad libitum.

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Peripheral cells counts. In separate experiments, peripheral blood (100 to 200 μL) was obtained by retro-orbital venipuncture twice a week (five mice per group) from days 4 to 27 after irradiation with or without transplantation. At any given time, peripheral cell counts were performed on randomly selected mice and were never repeated more than once every 2 weeks for any given animal. Leukocyte (white blood cell, WBC) counts were performed visually on a hemocytometer after lysis of erythrocytes. Hemoglobin levels and platelet counts were determined on a Coulter counter (Margency, France). Leukocyte differentials were determined by examination of Wright-Giemsa-stained slides of blood smears.

Spleen and marrow cellularity. From days 3 to 45 after BMT, randomly selected IL-1-pretreated and saline-treated surviving animals (five mice per group) were killed. Single-cell suspensions of splenocytes and marrow cells (harvested from the femurs and tibias) were obtained from individual mice. Nucleated cell numbers for each animal were determined visually on a hemocytometer.

Statistics. Means are reported ± standard error to the mean. All experiments were repeated three times with the exception of the post-transplant marrow cellularity and chimerism analysis, where the experiments were performed twice. Pooled data are presented except in Figs 1 and 6, where representative experiments are reported. Student’s t-tests were used to analyze differences in the means between groups (percent of donor cells, peripheral cell counts).

RESULTS

The administration of 3 μg of IL-1β IP to BALB/c mice 20 hours before a 700- to 800-cGy irradiation resulted in a 100% survival versus 0% in the radiation control group (P < 0.0001) (Fig 1A). One microgram of IL-1 was less efficient, with a survival rate of 70% (P < 0.005) (data not shown). Because of the excellent radioprotective effect of 3 μg of IL-1, a similar dose of IL-1 was used for all the subsequent experiments. IL-1 treatment before irradiation and transplantation of a limited amount of semiallogeneic BM (1 × 10E5 CB6F1 BM cells into BALB/c mice) also resulted in a survival rate close to 100%, whereas only 40% of the saline-treated transplanted mice survived (P < 0.005) (Fig 1B).

Because the survival rate of IL-1-pretreated nontransplanted mice was 100%, and because infusion of F1 marrow cells into an irradiated parent does not generate graft-versus-host disease, the favorable effect of IL-1 pretreatment on the survival of transplanted recipients was expected.

More surprising, but consistent with the results after IL-1 treatment and allogeneic T-cell-depleted BM, allogeneic engraftment levels measured in the spleen 2 months after BMT (Table 1) were not significantly different between IL-1-pretreated (56% ± 6%) and the surviving saline-treated recipients (66% ± 5%) (P = .25). The possibility that IL-1 could result in a slightly higher level of autologous reconstitution was not confirmed in the marrow, where the percent of donor-derived cells was similar in IL-1-pretreated mice (93% ± 4%) and saline-treated mice (95% ± 3%) (Table 1).
Table 1. Spleen and Marrow Chimerism Analysis in Surviving Animals 2 Months After BMT

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>IL-1</th>
<th>No. of Mice Analyzed</th>
<th>% of Donor-derived Cells (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>+</td>
<td>17</td>
<td>56 ± 6 (2-81) *</td>
</tr>
<tr>
<td>Spleen</td>
<td>–</td>
<td>10</td>
<td>66 ± 5 (25-79)</td>
</tr>
<tr>
<td>Marrow</td>
<td>+</td>
<td>8</td>
<td>93 ± 4 (70-100) *</td>
</tr>
<tr>
<td>Marrow</td>
<td>–</td>
<td>5</td>
<td>95 ± 3 (83-100)</td>
</tr>
</tbody>
</table>

* P > .05 (v mice not receiving IL-1).

Twice-weekly determinations of the peripheral cell counts in transplant recipients revealed that a single administration of IL-1 treatment before irradiation had profound effects on the three blood cell components (Fig 2). Leukocyte counts (Fig 2A) at day 4 were similarly low in both IL-1-treated and saline-treated mice. However, subsequently, the pattern of leukocyte recovery was significantly different between the two groups. Whereas the leukocyte counts in the control group remained very low until day 11 and slowly increased from then on, counts in the IL-1-treated animals significantly increased from days 4 to 7 (100 ± 10/mm³ vs 200 ± 20/mm³, P < .008) before decreasing from days 7 to 11 (135 ± 30/mm³, P < .01). Despite this secondary decrease, the leukocyte counts at day 11 stayed higher in the IL-1 group than in the saline group, where one must keep in mind that only 40% to 50% of the recipients are long-term survivors. This transient early increase of the leukocyte counts was repeatedly observed in three independent experiments. From day 11 on, leukocyte counts increased in both groups but with constantly higher counts in the IL-1-pretreated group.

The determination of leukocyte differentials revealed that the IL-1-induced “peak” in peripheral leukocyte counts observed between days 4 and 11 was mainly caused by the presence of mature granulocytes (140 ± 30/mm³) while, at the same time, their near absence (4 ± 2/mm³, P < .0001) was found in the peripheral blood of the control mice (Fig 3). These IL-1-induced peripheral granulocytes then significantly decreased from days 7 to 11 (45 ± 11/mm³, P < .004) before subsequently increasing again.

Platelet reconstitution after transplantation was also significantly accelerated by IL-1 pretreatment, with a significant difference versus saline-treated recipients as soon as day 11 (P < .002) and until day 18 (P < .04) (Fig 2B). In fact, the mean platelet count of the mice receiving IL-1 never decreased to lower than 100,000 ± 10,000/mm³ versus 40,000 ± 10,000/mm³ (P < .002) in surviving control mice. Erythrocyte reconstitution was also influenced by IL-1 treatment, although less significantly than for the leukocyte and platelet counts. Hemoglobin levels were identical in both groups until day 11, after which the hemoglobin reconstitution was accelerated in the IL-1-treated recipients, with significant differences between both groups at day 18 (11.3 g/100 mL ± 0.7 v 8.5 g/100 mL ± 0.6, P < .014) (Fig 2C).

We then examined the kinetics of splenocyte and marrow cell reconstitution after IL-1 pretreatment and BMT (Fig 4). IL-1 treatment did not prevent postirradiation splenocyte...
depletion, with similarly low numbers of cells at day 4 in the IL-1-treated and saline-treated recipients. In the marrow, the situation was quite different, with as early as day 4 an eightfold increase of cells in IL-1-treated mice versus control mice ($P < .0002$). Subsequently, the reconstitution of spleen and marrow cellularity were both significantly accelerated in the IL-1-treated mice. The IL-1-induced effects again predominated in the marrow, where 10-fold increases of the cellularity were observed both at days 7 and 11.

Chimerism analysis by fluorescein-activated cell sorter (FACS) analysis of spleen cells in individual mice early after BMT revealed low but increasing levels of donor-derived cells in IL-1-treated and saline-treated, with no significant difference between both groups (Table 2).

The important effects of a unique administration of IL-1 before irradiation and BMT and, in particular, the transient circulation of leukocytes early after transplant led us to wonder what was the influence of the bone marrow graft on the IL-1-induced hematopoietic reconstitution patterns. Because IL-1 pretreatment induces a survival rate greater than 90% with or without transplant, one could effectively examine the peripheral cell reconstitution without BMT. In fact, IL-1 treatment before irradiation without BMT also affected the reconstitution of leukocytes, platelets, and hemoglobin levels (Fig 5). However, the comparison between IL-1-pretreated and saline-pretreated irradiated mice was limited to the first 11 days after irradiation because of the 100% lethality of saline-pretreated animals.

Interestingly, IL-1 pretreatment also induced an early day 7 peak of circulating leukocytes (Fig 5A), predominantly constituted of mature granulocytes ($1 \times 10^6$ to $3 \times 10^6$ in saline-treated irradiated mice, $P < .006$) (Fig 6).

Table 2. Spleen Chimerism Analysis at Day 7 and 11 After BMT

<table>
<thead>
<tr>
<th>Day post-BMT</th>
<th>No. of Mice Analyzed</th>
<th>% of Donor-derived Cells (range)</th>
<th>No. of Donor-derived Cells (range) $\times 10^6$/Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 $+$</td>
<td>15</td>
<td>$5 \pm 1^*$</td>
<td>$3.4 \pm 0.6^*$</td>
</tr>
<tr>
<td>7 $-$</td>
<td>15</td>
<td>$3 \pm 1$</td>
<td>$1.5 \pm 0.8$</td>
</tr>
<tr>
<td>11 $+$</td>
<td>13</td>
<td>$20 \pm 2^*$</td>
<td>$78.3 \pm 17.6^*$</td>
</tr>
<tr>
<td>11 $-$</td>
<td>13</td>
<td>$26 \pm 4$</td>
<td>$59.2 \pm 10.6$</td>
</tr>
</tbody>
</table>

* $P > .05$ (v mice not receiving IL-1).

Fig 4. Spleen (A) and marrow (B) BM cell reconstitution in IL-1-pretreated (---) versus (O) saline-pretreated allogeneic BMT recipients. An average of 12 and 7 mice per group per time point were analyzed for spleen and marrow cellularity, respectively ($*: P < .05$).

DISCUSSION

As first reported by Neta et al., greater than 90% of IL-1-pretreated mice will survive an otherwise lethal dose of irradiation. This broad effect is thought to be mainly related to the capacity of IL-1 to promote hematopoiesis through several mechanisms: stimulation of various cells to produce hematopoietic growth factors (HGFs), upregulation of HGF receptors, direct effects on the survival of stem cells, and proliferative signals in synergy with other HGFs. On a theoretical basis, allogeneic BMT, unnecessary in terms of survival after IL-1 treatment and lethal irradiation, might be in an unfavorable situation compared with the autologous hematopoiesis and not be able to engraft and expand. Our data suggest that IL-1 treatment before allogeneic BMT does not enhance long-term marrow autologous reconstitution. Although BMT is not necessary for the survival of IL-1-treated BMT recipients, allogeneic marrow cells will engraft and overwhelmingly constitute marrow hematopoiesis 2 months after BMT. IL-1 pretreatment could therefore allow for the engraftment of very small amounts of allogeneic bone marrow cells that, in the absence of IL-1, would be insufficient to prevent a 100% recipient lethality. Preliminary experiments suggest that this is indeed the case.

In the spleen, the percentage of donor-derived cells appeared to be slightly lower in IL-1-pretreated mice than in surviving saline-treated mice. However, this difference, not observed in IL-1-pretreated mice receiving T-cell-depleted
IL-1 treatment before allogeneic BMT

BMT, did not reach statistical significance. The mean percentage of donor-derived cells was higher in the marrow than in the spleen of both IL-1-pretreated and saline-pretreated mice and is likely caused by the persistence of radioresistant autologous cells in the spleen and not in the marrow. The exact significance of this finding is presently unknown.

The present data demonstrate that IL-1 treatment profoundly affected hematopoietic recovery after irradiation with or without BMT causing not only an enhancing effect on leukocytes counts but also on platelet counts and hemoglobin levels. In fact, although endpoint hematopoiesis was obviously recipient derived without transplantation and mainly donor-derived with transplantation, IL-1 treatment affected all three blood cell compartments with or without BMT. A single administration of IL-1 before lethal irradiation, with or without BMT, has therefore potent multilineage effects on hematopoietic reconstitution. This finding is in agreement with the broad effects of this cytokine on hematopoiesis, particularly at the stem cell level. Also of interest, IL-1 pretreatment induced an early transient increase of granulocytes after irradiation independently of BMT. The numbers involved were modest, but the differences in granulocyte counts between IL-1-treated and saline-treated mice are statistically and clinically significant. Indeed, in a clinical setting, a granulocyte count over 100/mm$^3$ is clearly associated with a lower infectious risk than when this count is closer to 0. The considerable IL-1-induced survival advantage could be, at least partly, related to these circulating granulocytes. However, because IL-1 pretreatment also affected platelet and erythrocyte reconstitution, lesser anemia and/or bleeding disorders could have also prevented mortality. From a clinical standpoint, the effect of IL-1 on early granulopoiesis is clearly relevant because, in contrast to anemia or thrombocytopenia, severe post-transplant neutropenia cannot be prevented at the present time.

The fact that the increase in circulating granulocytes was early (maximum at day 7), transient, and occurred with or without allogeneic BMT suggests that after BMT these cells were from autologous origin. Spleen chimerism analysis did not indicate a significant increase in recipient-derived cells 7 days after BMT. Unfortunately, chimerism analysis of low numbers of circulating granulocytes early after transplantation is difficult to perform, and we have not yet been able to identify definitively the origin of these circulating cells early after allogeneic BMT.

How can one explain this IL-1-induced effect on granulocyte counts 1 week after a lethal irradiation? The early occurrence as well as the transient increase of these granulocytes suggests the involvement of a relatively mature hematopoietic progenitor cell. Induction of progenitor cells into a radioresistant S phase and/or mobilization of progenitors out of the marrow into the peripheral blood with consequently reduced radiosensitivity are two mechanisms through which IL-1 could confer radioprotection of progenitor cells. Indeed, IL-1 treatment is reported to induce a twofold increase in blood and spleen granulocyte-macrophage colony-forming cell (GM-CFC) numbers associated with a reduction in marrow GM-CFCs. Also, in contrast to marrow GM-CFCs,
the number of splenic GM-CFCs in S phase is increased by IL-1.\textsuperscript{19} Consistent with these results, recent data also suggest that the IL-1-mobilized peripheral stem cells comprise relatively mature (rhodamine +) clonogenic cells,\textsuperscript{20} whereas more primitive quiescent stem cells continue to reside in the marrow. Therefore, the early circulation of granulocytes after irradiation could result from the radioprotection (ie, IL-1-induced mobilization and cycling) of relatively mature progenitors before their subsequent maturation. The absence of a similar transient increase in platelet or hemoglobin levels could be because of the specificity of the radioprotected progenitors or more probably to the longer time-lag necessary for proliferation and maturation of these cell types, thus preventing a discernible transient increase of these autologous cells in the midst of an IL-1-enhanced hematopoietic reconstitution.

Many important questions involving IL-1 treatment before irradiation and BMT remain to be answered. Recently, Neta et al demonstrated that natural levels of IL-1 as well as tumor necrosis factor alpha (TNF-\alpha) contributed to radioresistance and that cooperative interaction of both cytokine was necessary to achieve successful radioprotection.\textsuperscript{21} The exact roles of TNF-\alpha as well as other cytokines such as granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, or IL-6\textsuperscript{22,23} on post-BMT hematopoietic reconstitution in IL-1-treated mice remain to be determined. A short-term persistence of autologous hematopoiesis after allogeneic BMT could have as a consequence a transient post-BMT state of mixed chimerism,\textsuperscript{24} and one can wonder if such a coexistence is not responsible, at least partly, for the subsequent successful engraftment. Repeated IL-1 administration to recipient mice after fully H-2-incompatible non-T-cell-depleted BMT is reported to increase the rate of wasting and early death, consistent with an exacerbation of GvHD.\textsuperscript{25} Also, post-BMT IL-1 inhibition by IL-1 receptor antagonist administration has been found to prevent GvHD.\textsuperscript{26} The effects of IL-1 before transplantation on GvHD and other important endpoints such as conditioning-related extrahematopoietic toxicity and the graft-versus-leukemia effect are currently being investigated in our laboratory.

Overall, our data suggest that the potent effect of IL-1 pretreatment on survival relates not only to an enhanced post-irradiation leukocyte, platelet, and erythrocyte reconstitution but also to the effective protection of a fraction of hematopoietic progenitors responsible for an early transient circulation of granulocytes. After allogeneic BMT, this last finding is evidence in favor of an early state of mixed chimerism before long-term allogeneic BM engraftment.

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