A Role for Manganese Superoxide Dismutase in Radioprotection of Hematopoietic Stem Cells by Interleukin-1

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Pretreatment with interleukin-1 (IL-1) has been shown to protect mice from the myelotoxicity associated with irradiation via a mechanism potentially mediated through the induction of the antioxidant enzyme manganese superoxide dismutase (MnSOD). In this study, we have compared the ability of IL-1 to induce MnSOD mRNA in murine bone marrow cells and human cell lines with its ability to protect these cells against the damaging effects of ionizing radiation. Bone marrow cells obtained from mice 6 hours after a single injection of IL-1 demonstrate a dose-dependent increase in the expression of MnSOD RNA. In this same study, IL-1 was also shown to be radioprotective when given to mice 20 hours before lethal irradiation. Similarly, in vitro treatment with IL-1 of bone marrow cells isolated from 5-fluorouracil-treated mice results in elevated levels of MnSOD RNA. Pretreatment with IL-1 also protected bone marrow long-term culture-initiating cells capable of reconstituting irradiated stromal cultures from an irradiation insult. Furthermore, IL-1-treated human bone marrow cells display both elevated MnSOD RNA and protein levels when compared with media controls. The human A375 melanoma, A549 adenocarcinoma, and factor-dependent TF-1 leukemia cell lines demonstrate low basal MnSOD RNA levels that increase following treatment with IL-1. For the A375 cells, this correlates with increased MnSOD protein expression and radioprotection by IL-1 using a colony assay. In contrast, the chronic myelogenous leukemic cell line, K562, displays a low basal MnSOD RNA level, and this RNA expression is not further increased by IL-1 treatment. In addition, these cells are comparatively radioresistant and are not further protected by IL-1 treatment. Finally, the Mo-7 cell line displays a low basal level of MnSOD RNA that correlates with a high sensitivity to irradiation and IL-1 pretreatment has no effect on MnSOD RNA levels. Our results indicate that increased radioprotection by IL-1 correlates with the induction of the antioxidant enzyme MnSOD and this induction may be an important factor in IL-1 radioprotection.

INTERLEUKIN-1 (IL-1) is a well-characterized cytokine that has been shown to play a major role in the mobilization of the immune response and the activation of inflammatory processes.4 There are two forms of the IL-1 molecule, both of which have been isolated and cloned.23 Although IL-1 alpha (IL-1α) and IL-1 beta (IL-1β) share only about 26% homology, they do share common receptors.1 Many of the in vitro and in vivo effects of IL-1 are involved in host defense, and it has been suggested that this cytokine also has homeostatic functions in such systems as normal bone turnover, sleep processes, and hematopoiesis.4

IL-1 has been shown to regulate hematopoiesis in a variety of ways. Many studies have shown that IL-1 is capable of inducing the in vitro production and release of colony-stimulating factors (CSFs) by fibroblasts,5,6 endothelial cells,7,14 and both primary and immortalized stromal cell cultures.6,9,11 In vivo, IL-1 treatment has also been shown to result in elevated levels of detectable serum CSF.12 IL-1 is able to enhance the proliferation of early hematopoietic progenitor cells in synergy with CSFs such as granulocyte CSF (G-CSF),13 IL-3,14 CSF-1,14 and granulocyte-macrophage CSF (GM-CSF).13 This activity was originally attributed to a factor, hemopoietin-1, that acts on very primitive multipotent cells and is present in the conditioned medium obtained from a human bladder carcinoma cell line.16,17 Purification and identification of this factor as IL-1 has led to the suggestion that IL-1 may act on an unresponsive stem cell population to induce CSF receptor expression.18

In vivo, IL-1 is able to stimulate neutrophil egress from the bone marrow19 and accelerate hematopoietic recovery in mice treated with myelosuppressive agents.13 Pretreatment of bone marrow cells with IL-1 has been reported to result in the protection of very early progenitor cells from the lethal effects of the chemotherapeutic agent 4-hydroperoxycyclophosphamide (4-HC).20 IL-1 has also been demonstrated to act as a protective agent for mice given lethal doses of ionizing radiation21 and to aid in hematopoietic recovery following sublethal irradiation.22 IL-1 as well as tumor necrosis factor alpha (TNF/α) are the only cytokines that demonstrate optimal protection when administered 20 hours before irradiation.23,24 IL-1 was reported to enhance the survival of irradiated animals given allogeneic bone marrow.25 Taken together, these results suggest that IL-1 acts to protect and aids in the recovery of host bone marrow cells and may accelerate engraftment of donor cells.

The action of IL-1 on bone marrow cells has been examined at the cellular level to determine the protective mechanisms operating. Studies have shown that IL-1 increases the number of colony-forming cells (CFCs) able to colonize the spleen.26 Using agents toxic to cells at specific stages of the cell cycle, Neta et al27 suggested that the protective effects of IL-1 may be because of its ability to induce progenitor cells to enter the radioresistant late S phase of the cell cycle. Other protective mechanisms may involve the induction of antioxidant enzymes such as manganese superoxide dismutase. IL-1 and TNFα have been reported to stimulate MnSOD expression at both the RNA and protein level.28,30 Furthermore, overexpression of MnSOD in human immunodeficiency virus (HIV)-infected human T-cell lines increases their survival when subjected to lethal irradiation.31 Because MnSOD is an important antioxidant enzyme, this study suggests that IL-1 may protect cells by increasing MnSOD expression.

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The levels of radiation-induced oxygen intermediate. We have looked at the induction of MnSOD RNA in murine bone marrow in response to IL-1 administration and compared its level with the in vivo and in vitro protection of these cells from irradiation. We have also studied the induction of MnSOD RNA and protein levels in human bone marrow cells and in a number of human cell lines in response to IL-1 and compared their ability to withstand irradiation after IL-1 pretreatment.

**MATERIALS AND METHODS**

**Materials.** B6D2F1 female mice (8 to 12 weeks of age) were obtained from Harlan Farms, Indianapolis, IN. Human recombinant IL-1β (specific activity 2 x 10^7 U/mg protein) was a kind gift of the DuPont Chemical Co, Delaware and diluted in Modified Eagle's Medium (MEM)–medium (GIBCO, Grand Island, NY) to the proper concentration. The cell lines K562, HL-60, A549, and A375 were obtained from the American Type Tissue Collection, Rockville, MD. The Mo-7 and TF-1 factor-dependent cell lines were obtained from Dr Alan Miller, University of Florida. Recombinant human IL-3 (specific activity 3.3 x 10^7 U/mg protein) and human GM-CSF (specific activity 7.1 x 10^6 U/mg protein) were kind gifts of Ortho Pharmaceutical Co, Raritan, NJ, and diluted in MEM–medium to the proper concentration. Fetal bovine serum (FBS) was obtained from Hyclone, Inc, Logan, UT.

**Human bone marrow cells.** Bone marrow samples were aspirated from the posterior iliac crest of healthy adult volunteers who had given written informed consent. Bone marrow mononuclear cells were obtained after centrifugation over Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) as previously described for use in all experiments.

**Animal studies.** Female B6D2F1 mice were used at 10 to 12 weeks of age. Mice were treated intraperitoneally with human recombinant IL-1β at concentrations of 125, 250, 500, or 1,000 ng/mouse or medium as a control. Six hours later, two mice from each group were killed and bone marrow cells collected for RNA studies. Twenty hours later, 8 to 10 mice from each group were irradiated at 9.5 Gy and kept in sterilized, filtered cages and given sterilized food and acidified drinking water for up to 8 weeks for survival studies. In addition, female B6D2F1 mice were also used at 8 to 10 weeks of age and given an intravenous injection of 5-fluorouracil (250 mg/kg) 2 days before bone marrow harvest (2-day post–5-FU bone marrow). Bone marrow cells were collected from the femurs, counted, and incubated with 100 ng/mL IL-1β for 6 hours for RNA studies or for 20 hours before being subjected to irradiation at 8 Gy using a gamma-cell 40 irradiator at 9.3 cGy/min. After washing, 5 x 10^5 bone marrow cells were plated on top of previously prepared marrow stromal cultures that were irradiated at 15 Gy to inactivate persisting hematopoietic cells as described below.

**Long-term bone marrow cultures.** Bone marrow cells were harvested from normal mice and 10^6 cells were placed in culture at 32°C in 25-cm² flasks in MEM–alpha supplemented with 25% horse serum and 2 x 10^{-3} mol/L hydrocortisone. After 3 to 4 weeks or when the cultures reached confluence, the flasks were irradiated using 15 Gy. Each flask was then reinoculated with 5 x 10^5 2-day post–5-FU bone marrow cells that were previously treated with either 100 ng/mL IL-1 or medium for 20 hours before irradiation at 8 Gy. At weekly intervals, one half of the media plus cells were removed and replaced with fresh culture medium. The total nonadherent cells obtained per flask were calculated for each group and recorded for 7 to 8 weeks of culture.

**Cell lines.** The myeloid leukemic cell line K562 was cultured in RPMI supplemented with 10% FBS, whereas the A375 and A549 cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO, Grand Island, NY) supplemented with 10% FBS. The MO-7 and TF-1 cell lines were grown in MEM–α supplemented with 10% FBS, 10^{-5} mol/L 2-mercaptoethanol, 2 mmol/L L-glutamine, and 10 U/mL human IL-3 or 10 U/mL human GM-CSF, respectively. All cultures were carried out at 37°C in a 5% CO₂ humidified atmosphere. The A375 and A549 cells grow as adherent monolayers and were passaged before reaching confluence by treatment at 37°C with 0.5% trypsin/0.02% EDTA. The cells were centrifuged (200g for 10 minutes) and resuspended at 1/6 dilution in fresh medium. For RNA analysis, the cell lines were incubated for different times ranging from 0 to 24 hours either with or without 100 ng/mL human IL-1β. The nonadherent cell lines were cultured in polypropylene tubes at 10^7 cells in 5 mL. The A375 and A549 cells were treated as confluent monolayers in 100-mm² culture dishes 1 day after passage. Cells were either centrifuged, in the case of suspension cultures, or the media were decanted from the adherent layers before RNA was isolated.

**In vitro cell protection studies.** Cell lines were cultured at 10^4 cells/mL either in the presence or absence of 100 ng/mL human recombinant IL-1β for 20 hours before irradiation at increasing dosages from 0 to 10 Gy. Irradiation was carried out at 9.3 cGy/min using a gamma-cell 40 irradiator at the described dose. After washing, the cells were diluted and plated in 0.35% agar in media, at 1 mL/plate. After 7 to 10 days, the plates were analyzed for the presence of colonies using an inverted microscope. Colonies greater than 20 cells were counted from each group of four replicate plates and the mean ± 1 SD determined.

**RNA isolation.** Total RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform method, as described by Chomczynski and Sacchi with modifications. Media were removed from the pelleted suspension cells after centrifugation or the adherent monolayer cells and 4 mol/L guanidinium thiocyanate, 25 mmol/L sodium citrate, pH 7.0, 0.5% sarcosyl, and 0.1 mol/L 2-mercaptoethanol were added to the cells. The monolayer cells were scraped off the plates and extracted with 0.1 vol of 2 mol/L sodium acetate, pH 4.0, an equal volume of water saturated phenol, and 0.2 vol of chloroform/isoamyl alcohol (48:1). The final suspension was cleared by centrifugation, the aqueous phase was removed, mixed with an equal volume of iso-propanol, and the RNA precipitated by centrifugation. The RNA pellet was resuspended in 100 μL of diethyl pyrocarbonate–treated water and precipitated twice more with ethanol. The pellet was dissolved in 50 μL diethyl pyrocarbonate–treated water and the amount of RNA determined by absorbance at 260 nm.

**Northern analysis.** For each experiment, equal amounts of RNA were loaded as determined by the absorbance reading. The RNA was denatured in 30 μL of formamide, formaldehyde, 3-[N-Morpholino]propane-sulfonic acid (MOPS) (pH 7), sodium acetate, EDTA, and ethidium bromide by heating at 65°C for 10 minutes. The RNA was fractionated on 1% agarose, 6% formaldehyde, MOPS buffered gel. The RNA was electrophoresed to a nylon membrane (Genescreen, Du Pont–New England Nuclear, Boston, MA) and covalently cross linked to the membrane with a UV light. The membrane was prehybridized in 0.45 mol/L sodium phosphate, 6% sodium dodecy salt, 1 mmol/L EDTA, and 1% bovine serum albumin (BSA) for 15 minutes. The membrane was hybridized for 16 hours at 60°C in the above hybridization solution with a 32P-labeled rat MnSOD, rat copper/zinc superoxide dismutase (Cu/ZnSOD), or cathepsin B cDNA isolated and characterized as previously described. The MnSOD and Cu/Zn-SOD cDNAs used as probes are 936 and 650 bp, respectively. The 32P-labeled cDNAs were products of the random primer extension method. The membranes were washed in 0.04 mol/L sodium phosphate, 0.1% SDS at 65°C, and subjected to autoradiography using an intensifying screen at -80°C for 16 to 24...
hours. The mRNA levels were quantitated by using a Bio Image Visage 60 video densitometry system (Millipore/Bio Image, Ann Arbor, MI). It should be noted that our rat MnSOD cDNA probe detects a single mRNA species in mouse cells, whereas human cells contain two mRNAs of 1 and 4 kb in length, as has been previously reported.24,31

Assay of MnSOD protein levels. A commercially available enzyme-linked immunosorbant assay (ELISA) kit for MnSOD that was developed by Kawaguchi et al27 was used throughout these experiments as previously described.38 Each sample for assay was obtained from 2 × 10^5 cells cultured for 20 hours in the presence or absence of 100 ng/mL IL-1. All cells were collected by centrifugation at 3,000 rpm for 5 minutes and resuspended in 0.5 mL phosphate-buffered saline (PBS). The cell suspension was then frozen at −80°C. After thawing at room temperature, each sample was sonicated. After centrifugation at 3,000 rpm for 15 minutes, the supernatant was diluted and assayed for MnSOD using the ELISA kit. Protein concentrations of each of the samples were determined using BSA as the standard in a BCA kit obtained from Pierce, Rockland, IL.

Statistical analysis. Statistical computations were performed using the Student's t-test for two means. Mouse survival rates were compared using a 2 × 2 contingency table analysis (X^2). Irradiation doses resulting in 95% inhibition of colony formation (LD95) were calculated for each experiment by extrapolating from a least square linear regression line relating the irradiation dose to the percentage of colony growth recovery. Statistical significance between the means of these concentrations was evaluated by the Student's t-test.

RESULTS

Protection of bone marrow from irradiation and induction of MnSOD RNA by IL-1. Animals were pretreated for 20 hours with the described IL-1 concentrations before lethal irradiation at 9.5 Gy. Figure 1 demonstrates that pretreatment with medium alone does not protect mice from a lethal dose of irradiation as 100% of the mice had died by the 18th day following irradiation. Pretreatment for 20 hours with 250 and 500 ng of IL-1 protected 100% of the mice from lethal irradiation, whereas pretreatment with 1 μg IL-1 showed only a 75% survival.

To evaluate the effects of IL-1 on MnSOD expression, MnSOD mRNA levels were detected from total RNA isolated from bone marrow cells treated with IL-1 for 6 hours at concentrations between 125 ng and 1 μg per animal (Fig 2). MnSOD levels were increased above base line at all of the IL-1 doses used and showed a gradual dose-dependent increase in MnSOD with a greater than threefold induction over basal levels seen at 1 μg IL-1.

Similarly, 2-day post–5-FU bone marrow cells pretreated for 20 hours with 100 ng/mL IL-1 prior to irradiation at 8 Gy were shown to promote an earlier recovery of cells when placed on top of irradiated long-term bone marrow stromal cultures (Fig 3). Higher numbers of nonadherent cells were collected from the flasks repopulated with IL-1–treated bone marrow cells when compared to media-treated cultures at all time points studied. When RNA was extracted from bone marrow cells isolated from 2-day post–5-FU–treated mice and cultured with and without 100 ng/mL IL-1 for varying lengths of time, a time-dependent induction of MnSOD RNA was observed (Fig 4).

The induction of MnSOD in human cell lines. All cell lines were incubated with and without 100 ng/mL IL-1 for 6 hours before cell lysis and RNA isolation. IL-1 was routinely used at 100 ng/mL in protection assays, and so to enable direct comparisons, 100 ng/mL was used for RNA induction in the different cell lines. Figure 5 shows the effects of IL-1 treatment on MnSOD RNA levels in five different human cell lines. When hybridized with a cDNA probe specific for MnSOD, bands at 4 and 1 kb were seen, as previously shown.24,31 The A375 cell line showed undetectable basal expression of MnSOD RNA in culture but RNA expression was induced following a 6-hour treatment with IL-1 (Fig 5, lanes 1 and 2). The level of induction was difficult to determine because of the undetectable basal level of MnSOD in the A375 cell line; however, conservatively, it is greater than 1.5-fold. Furthermore, IL-1 was also capable of inducing elevated MnSOD protein levels in A375 cells as measured using an MnSOD ELISA. Table 1 demonstrates that A375 cells incubated for 20 hours in the absence of IL-1 synthesized 120 ng MnSOD/mg protein, whereas A375 cells stimulated by IL-1 for 20 hours produced 970 ng MnSOD/mg protein. The Mo-7 cell line showed undetectable basal levels of MnSOD RNA as well; however, unlike the A375 cells, they were not induced by treatment with IL-1 (Fig 5, lanes 3 and 4). The K562 cells show higher basal levels of MnSOD RNA, and this level is not found to increase following treatment with IL-1 (Fig 5, lanes 5 and 6). TF-1 cells demonstrate detectable basal levels of MnSOD that also are induced by treatment with IL-1 (Fig 5, lanes 7 and 8). Finally, as previously reported,28 A549 cells show minimal basal levels of
MnSOD that are greatly induced when these cells are treated with IL-1 (Fig 5, lanes 9 and 10).

As a loading control, the membranes were reprobed with a Cu/ZnSOD probe29 that gives two bands at 0.7 and 0.9 kb. The levels of Cu/ZnSOD RNA expression were low in A375 and K562 cell lines but higher in the MO-7, TF-1, and A549 cell lines whether or not the cells were treated with IL-1.

**Effect of irradiation on colony formation.** Four different cell lines (A375, K562, TF-1, and Mo-7) were evaluated for colony growth after being subjected to different levels of irradiation. When each cell line was exposed to increasing doses of irradiation (2.5 to 8.0 Gy), a statistically significant dose-dependent suppression of colony growth was observed (data not shown). Mo-7 cells were considerably more sensitive to irradiation than A375 and TF-1 cells, whereas K562 cells were the most radioresistant of the cell lines tested.

As seen in Table 2, the LD95 value for K562 cells was significantly higher (P < .05) than the LD95 values for A375 and TF-1 cells that were higher than the LD95 value for Mo-7 cells. These LD95 values correlate with the basal amount of MnSOD RNA shown in Fig 5 for each of the different cell lines, respectively.

**Radioprotection of human cell lines by IL-1.** All cell lines were preincubated with IL-1 for 20 hours, which has previously been shown to result in maximum protection from irradiation and presumably allows time for both RNA induction and protein synthesis.30 The cells were then irradiated and cultured in agar for 7 to 10 days after which the colony number per plate was determined. Because the Mo-7 and TF-1 cell lines require the presence of specific hematopoietic growth factors such as IL-3 and GM-CSF, they were not evaluated for protection from irradiation by IL-1. A549 cells, which demonstrate a significant induction of MnSOD RNA by IL-1, were also not evaluated because they could not be grown in agar culture. Figure 6 shows one of three similar experiments in which pretreatment of K562 cells with IL-1 was shown to have no effect on the growth characteristics in both the unirradiated and irradiated groups. No protective effects of IL-1 were seen for K562 cells at 6 or 8 Gy (Fig 6) nor when lower radiation doses were used (data not shown). In contrast to these results, Fig 7 demonstrates in one of three similar experiments that A375 cells were protected from irradiation by prior incubation with IL-1. When A375 cells were pretreated with IL-1 in the absence of irradiation, no effect on proliferation was observed, although higher levels...
of IL-1 have been previously reported to inhibit the growth of these cells.\textsuperscript{30,39,40} Figure 7 also shows that irradiation at either 6 or 8 Gy significantly reduced the number of colonies seen when compared to the unirradiated groups. Pretreatment of A375 cells with IL-1 in the irradiated groups results in the protection of a significant proportion of the colony-forming cells, with five times more colonies appearing after 6 Gy and seven times more colonies seen after 8 Gy from the IL-1 group when compared with the media-treated group. Again, both of the cell lines examined showed differences in their sensitivities to radiation that compare favorably with their endogenous MnSOD RNA levels. For example A375 cells, which express low or undetectable levels of MnSOD RNA (Fig 5), are very sensitive to irradiation, with only 0.7% of the colonies being recovered following 6 Gy (Fig 7). In contrast, K562 cells, which express elevated levels of MnSOD RNA (Fig 5), are less sensitive to irradiation with almost 3% of the colony-forming cells recovered following 6 Gy of irradiation (Fig 6).

\textit{MnSOD RNA and protein levels from human bone marrow cells.} Figure 8 shows the effects of a 4-hour incubation with 10 and 50 ng/mL IL-1 on MnSOD RNA induction in human bone marrow cells. An IL-1 dose-response effect is seen with enhanced MnSOD RNA levels above the 4-hour media control observed. Similarly, Table 1 shows the effects of a 20-hour incubation with IL-1 on MnSOD protein levels obtained from human bone marrow cells. The basal MnSOD protein level for human bone marrow cells is 1,850 ng/mg protein;
however, after treatment with IL-1, the MnSOD protein level is elevated about 2.5-fold to 5,880 ng/mg protein.

**DISCUSSION**

Previous studies have shown that IL-1 administration will protect mice from both lethal and sublethal doses of ionizing radiation.20,21 This protective action occurs when IL-1 is injected into mice 1 day before exposure to total body irradiation. In this report, we have confirmed that IL-1 administration to mice 20 hours before lethal irradiation will bring about a significant increase in survival. In addition, we have shown that preincubation of bone marrow cells with IL-1 protects early long-term culture-initiating cells from an irradiation insult and that these cells are capable of reconstituting irradiated stromal cultures. These experiments suggest that early progenitors, including stem cells, are protected from irradiation by IL-1.

The precise mechanism by which irradiation destroys marrow stem cells is not clear. However, because the majority of stem cells are normally quiescent and in G0 or a long G1, it is possible that ionizing radiation damages stem cells by causing formation of free radicals that induce peroxidation of lipids and strand breaks in DNA. MnSOD has been implicated in the protection against oxidative stress by a number of studies using cell lines that have been engineered to overexpress MnSOD.31,42,43 From these studies, Wong et al42 have proposed that the induction of MnSOD by IL-1 protects early long-term culture-initiating cells from an irradiation insult and that these cells are capable of reconstituting irradiated stromal cultures. These experiments suggest that early progenitors, including stem cells, are protected from irradiation by IL-1.

Table 1. Effect of IL-1 on MnSOD Protein Levels in Human Bone Marrow Cells and A375 Cells as Measured by ELISA

<table>
<thead>
<tr>
<th>Cell Populations</th>
<th>Mean Value of MnSOD ng/mg Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human bone marrow + media</td>
<td>1,850</td>
</tr>
<tr>
<td>Human bone marrow + IL-1</td>
<td>5,880</td>
</tr>
<tr>
<td>A375 cells + media</td>
<td>120</td>
</tr>
<tr>
<td>A375 cells + IL-1</td>
<td>970</td>
</tr>
</tbody>
</table>

**Fig 6. Protection of K562 cells from irradiation.** K562 cells were incubated with and without 100 ng/mL IL-1 for 20 hours before irradiation at 0, 6, or 8 Gy. After washing, the cells were plated in 0.7% agar and the number of colonies appearing after 7 to 10 days of culture recorded. (m) Colonies 10,000 cells.

**Table 2. Dose of Irradiation Resulting in 95% Reduction of Colony Formation From Different Cell Lines**

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>MnSOD RNA *</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt;(Gy)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>K562</td>
<td>+</td>
<td>5.8</td>
</tr>
<tr>
<td>TF-1</td>
<td>±</td>
<td>4.7‡</td>
</tr>
<tr>
<td>A375</td>
<td>–</td>
<td>4.1†</td>
</tr>
<tr>
<td>Mo-7</td>
<td>–</td>
<td>2.6†</td>
</tr>
</tbody>
</table>

* Relative MnSOD RNA basal values from Fig 5.
† The LD<sub>50</sub> values are expressed as the arithmetic mean of the irradiation dose resulting in 95% inhibition of colony formation calculated for each experiment by extrapolating from a least square linear regression line relating irradiation dose to the percentage of recovery of colony formation.
‡ Statistically different (P < .05) from LD<sub>50</sub> value for K562 cells when compared with Student’s t-test for paired data (two-tailed values).

**Fig 7. Protection of A375 cells from irradiation.** A375 cells were incubated with and without 100 ng/mL IL-1 for 20 hours before irradiation at 0, 6, or 8 Gy. After washing, A375 cells were plated in 0.7% agar and the number of colonies appearing after 7 to 10 days of culture recorded. (m) Colonies 10,000 cells.
MnSOD enzyme activity than the resistant clone, no correlation could be seen between MnSOD and sensitivity to the antiproliferative effects of IL-1.

Although the present study as well as studies by others suggest that MnSOD may be responsible for the protection from radiation seen with IL-1, other free radical scavengers may also be involved. It is thought that an increase in the level of catalase may be important to remove the hydrogen peroxide produced by MnSOD, and elevated glutathione peroxidase concentrations may also contribute to full radioprotection. The hydroxyl radical scavenger metallothionein has also been shown to be induced by IL-1, and it has been suggested that it may act in a complementary manner with SOD to enhance protection against free radicals. Further studies are needed to verify the role of each of these factors in radioprotection.

Treatment of neoplastic conditions often involves the use of radiation therapy or cytotoxic drugs. Hematopoietic tissue is known to be highly sensitive to the damaging effects of such agents, which result in decreased bone marrow and immune function. Protection of bone marrow stem cells by IL-1 is a possible way of reducing the myelotoxicity associated with cancer therapy. In clinical trials, IL-1 appears to promote recovery from leukopenia and neutropenia, caused by cytotoxic agents, and to increase platelet counts. It also has been suggested that IL-1 expands the hematopoietic progenitor pool and that pretreatment with IL-1 will protect early hematopoietic progenitors from the lethal effects of the chemotherapeutic agent 4-HC.

The present study shows that in addition to the ability of IL-1 to induce growth factors and augment proliferation that may aid in hematopoietic repopulation, the cytokine also induces RNA and protein synthesis for the antioxidant enzyme MnSOD in certain cell populations. We have been able to correlate this induction with increased resistance to the damaging effects of ionizing radiation. However, direct proof of the role of MnSOD in radioprotection will require either inhibition of MnSOD resulting in increased susceptibility to irradiation and/or inserting the MnSOD gene in susceptible cells to make them radiosensitive. These types of studies are currently underway.

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A role for manganese superoxide dismutase in radioprotection of hematopoietic stem cells by interleukin-1

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