Prevention of Acute Chemotherapy-induced Death in Mice by Recombinant Human Interleukin 1: Protection from Hematological and Nonhematological Toxicities


ABSTRACT

Previous studies have demonstrated that interleukin 1 (IL-1) can protect most mice from the acute lethal toxicity mediated by high doses of radiation and/or some chemotherapeutic drugs. The results presented herein demonstrate that the pretreatment of mice with recombinant human interleukin 1α (rhIL-1α) protects mice from the lethal effects of several myelotoxic chemotherapeutic drugs, including 5-fluorouracil (5FUra), cyclophosphamide, cis-diammine(1,1-cyclobutane dicarbonyl)platinum(II), and 1,3-bis-(2-chloroethyl)-1-nitrosourea. However, pretreatment with rhIL-1α was not effective against the acute lethal toxicity generated by doxorubicin and cisplatin. The chemoprotective effects appear to be at least partially due to myeloprotection/restoration, since the recovery of myeloid colony-forming units and the total cellularity in the bone marrow and spleen were accelerated in the rhIL-1α-pretreated mice. However, the chemoprotective effects of rhIL-1α are apparently not limited to myeloprotection, since pretreatment with rhIL-1α protects mice against the lethal toxicity of both 5FUra and cyclophosphamide, yet bone marrow transplants rescue mice treated with 5FUra but not those treated with cyclophosphamide. The chemoprotective effects of rhIL-1α may be at least partially indirect, since the efficacy of chemoprotection by rhIL-1α is reduced in athymic mice, and interleukin 6, but not tumor necrosis factor α, can substitute for rhIL-1α in chemoprotection from 5FUra.

INTRODUCTION

Myelosuppression is the dose-limiting toxicity for most anticancer chemotherapeutic agents (1). The resulting granulocytopenia renders many patients susceptible to opportunistic infections that are often lethal (2). Agents that protect the hematopoietic and lymphoid systems from the suppressive effects of radiation or chemotherapy would be beneficial for two reasons: first, the side effects induced by a standard therapy regimen might be ameliorated; second, the higher therapeutic doses that could be administered might result in increased antitumor efficacy (3).

IL-1β is a multifunctional cytokine that regulates immune responses, inflammation, and hematopoiesis (4–8). Data from several laboratories have documented the protective effects of IL-1 in mice on the lethal toxicity induced by high doses of radiation (9, 10) or by the chemotherapeutic drugs Cy and 5FUra (11, 12). Futami et al. (12) demonstrated that a 7-day pretreatment with IL-1 protected mice bearing a murine renal cancer from the acute toxicity of high doses of Cy, and this translated into increased antitumor effects in vivo. Posttreatment with IL-1 in this model was not chemoprotective.

The mechanism(s) underlying the radioprotective or chemoprotective effects of IL-1 are not yet clearly defined. It has been suggested that the radioprotective and chemoprotective effects of IL-1 treatment are related to its ability to accelerate the recovery of critical hematopoietic progenitor cells in bone marrow (9) and an associated increase in WBC (13). Moore and Warren (13) have recently reported that the combined treatment of mice with IL-1 and G-CSF decreased the severity and duration of the neutropenia that followed the administration of 5FUra (13).

It remains unclear whether the beneficial protective effects of IL-1 are restricted to the hematopoietic system or whether IL-1 also protects against other chemotherapy-induced organ toxicities. It remains to be determined whether these effects are direct or rather are mediated by the stimulation of other cells and the subsequent release of other cytokines, such as IL-6 or TNF, that are known to have important effects on the immune and hematopoietic systems (14–17). IL-1 has been shown to stimulate fibroblasts, endothelial cells, and bone marrow stromal cells to produce G-CSF and GM-CSF (18–20) and to induce colony-stimulating activity in the serum of mice following treatment in vivo (21). In vivo treatment with IL-1 also induces the secretion of both IL-6 and TNF (22–24). The present studies were designed to determine the scope of IL-1 chemoprotection for several of the major chemical classes of chemotherapeutic drugs and to begin assessing the mechanisms for those effects. The 5FUra- and Cy-induced lethal toxicity models were selected as the focus of these studies since 5FUra-induced death is mainly due to bone marrow failure and Cy-induced death is due to a combination of myelotoxic and nonmyelotoxic effects.

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The abbreviations used are: IL-1, interleukin 1; Cy, cyclophosphamide; 5FUra, 5-fluorouracil; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-6, interleukin 6; TNF, tumor necrosis factor; G-CSF, granulocyte colony-stimulating factor; rhIL-1α, recombinant human IL-1α; rhIL-6, recombinant human IL-6; DDP, cis-diammine-dichloroplatinum; carboplatin, cis-diammine(1,1-cyclobutane dicarbonyl)platinum(II); BCNU, 1,3-bis-(2-chloroethyl)-1-nitrosourea; DOX, doxorubicin; NMS, normal mouse serum; CFU, colony-forming units; CFU-c, single lineage monocyte, myeloid, or myelomonocytic colonies; BMT, bone marrow transplantation; rmTNF, recombinant mouse tumor necrosis factor; LD50-90, 90–100% lethal dose.

5 The abbreviations used are: IL-1, interleukin 1; Cy, cyclophosphamide; 5FUra, 5-fluorouracil; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-6, interleukin 6; TNF, tumor necrosis factor; G-CSF, granulocyte colony-stimulating factor; rhIL-1α, recombinant human IL-1α; rhIL-6, recombinant human IL-6; DDP, cis-diammine-dichloroplatinum; carboplatin, cis-diammine(1,1-cyclobutane dicarbonyl)platinum(II); BCNU, 1,3-bis-(2-chloroethyl)-1-nitrosourea; DOX, doxorubicin; NMS, normal mouse serum; CFU, colony-forming units; CFU-c, single lineage monocyte, myeloid, or myelomonocytic colonies; BMT, bone marrow transplantation; rmTNF, recombinant mouse tumor necrosis factor; LD50-90, 90–100% lethal dose.
MATERIALS AND METHODS

Mice. Euthymic or athymic male BALB/c mice were obtained from the Animal Production Area, National Cancer Institute–Frederick Cancer Research and Development Center (Frederick, MD) and maintained under specific pathogen-free conditions. Experiments were performed using mice at 6–10 weeks of age. Mice were routinely maintained at 5/cage inside filter bonnets in an isolation room, under specific pathogen-free conditions.

Agents. rhIL-1α (specific activity, 2 × 10^7 units/mg protein) was generously provided by Dainippon Pharmaceutical Company (Osaka, Japan), rhIL-6 was kindly supplied by the Ajinomoto Pharmaceutical Company (Kawasaki, Japan), and mouse TNF (specific activity, 2.6 × 10^7 units/mg) was kindly supplied by Dr. M. Palladino (Genentech, Inc., South San Francisco, CA). Cy was purchased from the Sigma Chemical Co. (St. Louis, MO); SFUra from Lympho Med, Inc. (Rosemont, IL); and DDP, carboplatin, and BCNU from Bristol Laboratories (Syracuse, NY); and DOX from the Sigma Chemical Co.

Detection and Quantitation of Serum Cytokines. Pooled sera from 5 BALB/c mice/group were assayed as follows for the presence of various cytokines. IL-6 was assayed by enzyme-linked immunosorbent assay (Endogen, Inc., Boston, MA), and the data are presented as pg/ml. Both CSF and TNF were detected by bioassay. TNF activity is expressed as units/ml, where 1 unit results in 50% growth inhibition of actinomycin D-treated mouse L929 cells (25). CSF activity was measured by proliferation of the 32D CL3 progenitor cell line (26), whereby the number of biological units of CSF activity was determined to be the reciprocal of the serum dilution that resulted in 50% maximal stimulation. This assay detects proliferative effects of IL-3, GM-CSF, and G-CSF, but not CSF-1. Thus in the absence of specific neutralizing antibody studies, we have specified only that CSF activity is induced.

Administration of IL-1 and Anticancer Drugs. The rhIL-1, rhIL-6, and rmTNF-α were diluted to the desired concentration in H2O containing 0.5% NMS as a carrier before the i.p. injection of 0.3 ml/mouse. Cy, DDP, carboplatin, and DOX were diluted to the desired concentrations in H2O, while SFUra was diluted in phosphate-buffered saline and BCNU was diluted in 0.9% NaCl.

Soft Agar Colony-forming Assay. Bone marrow cells were flushed from the femurs and tibiae of mice by irrigation with RPMI 1640. The cells obtained from the femurs and tibiae were pooled from 3–4 mice/group, and single cell suspensions were made by passing the cells through a nylon mesh after vigorous pipeting. The cells were then washed twice and resuspended in minimum essential medium α (Gibco, Grand Island, NY) containing 10% fetal calf serum (Sterile Systems, Logan, UT), 1% L-glutamine (Cellgro, Washington, DC), 1% penicillin-streptomycin mixture (Cellgro), minimum essential medium vitamins, and sodium bicarbonate. Spleens were aseptically removed from mice and mechanically dissociated with forces. Single cell suspensions were obtained by passage through a mesh screen after vigorous pipeting and then washed twice and resuspended in the same media as described for the suspension of bone marrow cells. Bone marrow or spleen cells were plated at 1 × 10^5 and 0.4 × 10^5 or 1 × 10^6 cells/ml, respectively, in 35-mm Lux Petri dishes (Miles Laboratories, Inc., Naperville, IL) containing a 1-ml mixture of minimum essential medium α, 20% fetal calf serum, 1% L-glutamine, 2 × 10^-4 m hemin (Sigma Chemical Co.), 5 × 10^{-5} m 2-mercaptoethanol (Sigma Chemical Co.), 4–6 units/ml erythropoietin (AMGEN, Thousand Oaks, CA), 100 units/ml IL-3, and 0.005 mg/ml gentamicin (National Cancer Institute–Frederick Cancer Research and Development Center Central Media Laboratory) in 0.35% Seaplaque agarose (FMC Bioproducts, Rockland, ME). Dishes were incubated at 37°C in 5% CO2 and screened for CFU 10 days later. Multipotent colonies containing granulocyte, erythroid, megakaryocyte, and macrophage lineages are designated as CFU-GEMM, while monocyte, myeloid, and myelomonocytic colonies are designated as CFU-c.

Bone Marrow Transplantation. Mice were treated with lethal doses of Cy and SFUra, and then 4, 24, or 48 h later 10–15 × 10^6 bone marrow cells obtained from normal mice were injected into the caudal vein of the chemotherapy-treated recipient mice in a volume of 0.4 ml. This number of cells has been shown to completely reconstitute the hematopoietic system of lethally irradiated mice (27).

Statistical Analysis. Differences in the number of CFU-c colonies between various treatment groups were analyzed using Student’s t test. Differences in survival duration between various treatment groups were analyzed using Fisher’s χ² test.

RESULTS

Protective Effects of rhIL-1α Pretreatment against Acute Lethal Toxicity of High Doses of Cy and SFUra. Previous data from our laboratory demonstrated that pretreatment of mice for 7 days with rhIL-1α (500 ng/mouse/day i.p.) could protect 80–100% of the mice from the subsequent administration of a lethal dose of Cy (LD90-100). However, many mice that were successfully protected from the acute toxicity of Cy subsequently died after day 50 because of progressive pulmonary inflammation and fibrosis (12). In contrast, identical experiments performed with SFUra also demonstrated effective chemoprotection, in the absence of late pulmonary toxicity.

The data presented in Fig. 1 extend these observations for the SFUra model. Fig. 1A shows that the standard rhIL-1α pretreatment regimen protected mice (100% protection) from death induced by a SFUra dose of 280 mg/kg (LD90). This chemoprotection by rhIL-1α allowed the dose of SFUra to be
increased up to 400 mg/kg. The chemoprotective effects of rhIL-1α were diminished when doses of 5FUra ≥425 mg/kg were used (data not shown). In contrast to the previous observations for IL-1-mediated chemoprotection against Cy (12), no late toxicity was observed in the mice protected against 5FUra by rhIL-1α during the 3-month observation period (data not shown). Thus experiments investigating the chemoprotection by rhIL-1α for 5FUra were terminated on day 60.

The chemoprotective effects of rhIL-1α for 5FUra were dose-dependent with a daily dose of 500 ng rhIL-1α achieving maximal protection (90% survival), while a daily dose of 50 ng/mouse rhIL-1α protected only 20% of the mice that received 280 mg/kg 5FUra (Fig. 1B). This dose response for 5FUra chemoprotection by rhIL-1α is similar to that which we reported previously for Cy (12).

Effect of rhIL-1α Pretreatment on Bone Marrow and Spleen 

CFU-c. IL-1 has been reported to stimulate hematopoiesis in vivo (29, 30), and previous studies have shown that pretreatment of mice for 7 days partially protected against Cy-induced neutropenia and induced a pronounced extramedullary hematopoiesis in the spleen with no overall increase in total bone marrow CFU-c (12). Thus since 5FUra is even more selective with regard to myelotoxicity than is Cy, we speculated that at least some of the IL-1-induced chemoprotection against 5FUra could be attributed to myelopoiesis. The results shown in Fig. 2A demonstrated that the nadir in bone marrow CFU-c in both groups (5FUra-treated alone or rhIL-1α plus 5FUra-treated mice) occurred 2–5 days after 5FUra injection, and rhIL-1α pretreatment did not prevent this nadir [2 ± 0 (SD) versus 2 ± 2 and 3 ± 0 versus 2 ± 0 on days 2 and 5, respectively]. However, a more rapid recovery in bone marrow CFU-c was observed on days 7, 9, and 12 for mice pretreated with rhIL-1α versus those treated with only 5FUra [10 ± 1 versus 3 ± 1 (P ≤ 0.03), 216 ± 60 versus 52 ± 11 (P < 0.01), and 572 ± 43 versus 328 ± 18 (P < 0.005) × 10^3/mouse, respectively]. Also, the depression in the total number of bone marrow cells recovered faster in the rhIL-1α-pretreated group (Fig. 2A).

Pretreatment with rhIL-1α increased splenic hematopoiesis (Fig. 2B, day 0). However, this rhIL-1α-induced increase in extramedullary hematopoiesis was eliminated by the subsequent administration of a lethal dose of 5FUra such that no difference in the nadir of splenic CFU-c was observed between the rhIL-1α-pretreated and control mice. However, the 5FUra-induced leukopenia was more transient in mice that had been pretreated with rhIL-1α, since overall cellularity returned to about 50% and 120% of normal (control, day 0) levels by day 9 in the bone marrow and spleen, respectively. Conversely, the 5FUra alone control group remains at about 3% and 30% of control on day 9 in the bone marrow and spleen, respectively. This result is similar to what was previously reported for chemoprotection from Cy (12).

Effects of Bone Marrow Transplantation on 5FUra and Cy-induced Lethal Toxicity. Although the most common dose-limiting toxicity of many anticancer agents is myelotoxicity, toxicity to other organs, such as gut, bladder, kidney, and heart, can play an important role in the acute death induced by these agents. Thus, studies were performed to determine whether the chemoprotective effects of rhIL-1α were limited to myeloprotection. If the effects of the rhIL-1α pretreatment were limited to myeloprotection, then we could expect that a BMT shortly after the administration of chemotherapy would salvage these mice as efficiently as the pretreatment with rhIL-1α. BMT has been shown to rescue lethally irradiated mice (28). Normal bone marrow cells (10–15 × 10^6) were transfused on day 0 (4 h after the injection of either 5FUra or Cy) and on days +1 and +2 (Fig. 3A). All of the mice treated with either 280 or 325 mg/kg 5FUra alone died within 2 weeks, while 90–100% of the mice given a subsequent BMT following 5FUra treatment survived (P < 0.01). In contrast, after a single dose of 390 mg/kg Cy (Fig. 3B), 90% of the mice died within 12 days, and BMT following Cy treatment rescued only 10% of the mice, while pretreatment with rhIL-1α followed by Cy protected most mice from acute fatal toxicity (P < 0.03). As expected from previous studies (12), some of the mice that were chemoprotected by rhIL-1α from Cy began to die at about day 55. These data suggest that the dose-limiting toxicity of 5FUra is myelotoxicity, while for Cy additional toxicities become dose-limiting when the marrow is protected. Furthermore, the results suggest that since rhIL-1α pretreatment protects against Cy-induced acute toxicity, the mechanism of its action is broader than simple myeloprotection.

Effect of rhIL-1α Pretreatment on Lethal Toxicity Induced by Other Anticancer Agents. The chemoprotective effect of
CHEMOPROTECTIVE EFFECTS OF rhlL-1

rhIL-1α pretreatment was also tested with various other anticancer drugs, some with relatively selective hematological toxicity (BCNU, carboplatin) and some with dose-limiting toxicities related to other organs (DDP nephrotoxicity and DOX cardiotoxicity). As seen in Fig. 4A, when mice were treated with acutely lethal doses of carboplatin, a DDP analogue devoid of nephrotoxicity but which is myelotoxic (31, 32), 100% of the mice survived to day 60 after a dose of 140 mg/kg in the rhIL-1α-pretreated group versus 30% survivors in the carboplatin alone group (P < 0.03). Increasing the dose of carboplatin to 180 mg/kg resulted in 50% versus 0% survivors at day 60 in the rhIL-1α-pretreated versus control groups, respectively (P < 0.05). In contrast, as seen in Fig. 4B, mice treated with two different doses of DDP were not protected by pretreatment with rhIL-1α, while the same treatment was effective in preventing 5FUra-induced acute toxicity. These results suggest that rhIL-1α pretreatment is most effective in protecting mice from drugs that are mainly myelotoxic, but not from drugs whose dose-limiting toxicities are nephrotoxicity or cardiotoxicity.

Effects of rhIL-1α Pretreatment on 5FUra-induced Lethal Toxicity in Athymic Mice. IL-1 has profound effects on T-cells, and T-cells produce a number of cytokines that stimulate hematopoiesis. Therefore, to determine whether the protective effects of rhIL-1α could be mediated in part indirectly by T-cells, several chemoprotection experiments were performed in athymic mice. In contrast to the ability of rhIL-1α to efficiently chemoprotect in euthymic mice, rhIL-1α was less effective in athymic BALB/c mice. As shown in Fig. 6A, the LD₉₀ for 5FUra in athymic mice is about 260 mg/kg, which is somewhat lower than the 280-300 mg/kg in euthymic mice. Pretreatment with rhIL-1α was able to rescue only 60% (P < 0.03) of the athymic mice that were treated with 260 mg/kg of 5FUra, and increasing the dose of 5FUra further decreased the IL-1-mediated protection. These data suggest that some of the rhIL-1α-mediated chemoprotective effects are T-cell dependent but that other intermediates may also contribute.
CHEMOPROTECTIVE EFFECTS OF rhlL-1

Fig. 5. Effect of rhIL-1α pretreatment (500 ng/mouse/day) on lethal toxicity induced by DOX and BCNU. A, BALB/c mice (10 mice/group) were pretreated for 7 days with H2O plus 0.5% NMS (○, △) or rhIL-1α (●, ▼) and 24 later were treated with 12 mg/kg DOX (□, ◆), 14 mg/kg DOX (△, ▼), or 16 mg/kg DOX (●, ▽). B, BALB/c mice (10 mice/group) were pretreated for 7 days with H2O plus 0.5% NMS (○, △) or rhIL-1α (●, ▼) and 24 later were treated with 50 mg/kg BCNU (△, ▼), 60 mg/kg BCNU (○, ◆), or 70 mg/kg BCNU (△, ▽).

Fig. 6. Effect of rhIL-1α treatment on acute SFUra toxicity in athymic BALB/c mice. Athymic BALB/c mice (10 mice/group) were treated i.p. with H2O plus 0.5% NMS (○, △) or rhIL-1α (500 ng/mouse; ●, ▼) daily for 7 days before the i.v. administration of different doses of SFUra. A, 230 mg/kg SFUra (△, ▼) and 260 mg/kg SFUra (○, ◆). B, 280 mg/kg SFUra (△, ▼), 325 mg/kg SFUra (○, ◆), 5FU, SFUra.

Effects of Pretreatment with rhIL-6 and rmTNF Treatment on 5FUra-induced Acute Toxicity. It has been reported that the administration of rhIL-1α to mice results in the rapid appearance of CSFs, IL-6, and TNF in the circulation and that these induced cytokines might in turn be at least partially responsible for the effects induced by IL-1. Thus, several studies were performed to determine whether some of these various cytokines were being induced in detectable amounts under the injection protocols used in our study. The data shown in Table 1 demonstrate that in two separate experiments the i.p. injection of rhIL-1α is rapidly followed by the induction of serum IL-6 at concentrations up to >1250 pg/ml, as previously reported (22). Similarly, the injection of rmTNF-α is followed by the appearance of a small amount of serum IL-6. Both rhIL-1α and rmTNF-α induce detectable serum CSF activity, although the nature of the specific CSFs (IL-3, GM-CSF, or G-CSF) induced by each cytokine remains undetermined. The administration of rhIL-1α also resulted in the induction of low but detectable levels of TNF activity.

IL-6 has been shown to stimulate the recovery of the immune and hematopoietic systems after the administration of a sublethal dose of SFUra and to prolong the survival of mice treated with lethal doses of SFUra (33). TNF has been shown to be radioprotective, albeit to a lesser extent than IL-1 (34). As can be seen in Fig. 7A, pretreatment with rmTNF-α does not protect against the acute toxicity of SFUra (280 mg/kg) at any of the different doses and schedules tested. The data in Fig. 7B show that pretreatment of mice with rhIL-6 (5 μg/mouse i.p. twice a day for 7 days) protected 70% (P < 0.02) of the mice that were subsequently treated with a lethal dose of 5FUra. Lowering the dose of rhIL-6 to 1 μg/mouse twice a day decreased the protective effect to 40% versus 10% in the control group. These results suggest that IL-6 induced by rhIL-1α could contribute to the chemoprotective effects of rhIL-1α, at least for acutely toxic doses of 5FUra.

DISCUSSION

The results reported in this paper demonstrate that the pretreatment of mice with rhIL-1α for 7 days is effective in preventing death in mice that were subsequently treated with lethal doses of several different antitumor agents. The precise mechanism(s) for the chemoprotective effects of IL-1 pretreatment are now under investigation. A possible myeloprotective and/or myelorestorative mechanism has been proposed based on the well-known effects of IL-1 on hematopoiesis (29, 35). IL-1 has...
been shown to be an important proliferative signal for bone marrow cells, and Neta et al. (35) showed that the i.p. administration of IL-1 resulted in the enhanced cycling of bone marrow cells and that IL-1 induced a 2-fold increase in the proportion of cells sensitive to hydroxyurea, a drug that is lethal to cells in S phase and arrests cells in the late G₂ phase of the cell cycle. Multiple i.p. doses of IL-1 pretreatment were able to protect from Cy-induced lethal toxicity, and this protection correlated with an augmentation of several hematological parameters (in the bone marrow and spleen CFU-c) and with a more rapid recovery in WBC and neutrophil counts (11). Fibbe et al. (36) also reported that the administration of a single dose of IL-1 accelerated the recovery of peripheral blood neutrophils in Cy-treated mice. Moore and Warren (13) demonstrated that posttreatment with multiple doses of IL-1 alone or in combination with G-CSF reduced the nadir and the duration of neutropenia with a faster recovery of peripheral blood neutrophils in C3H/HeJ mice that had been treated with a sublethal dose of SFUra. It remains uncertain whether pretreatment with rhIL-1α acts in protecting critical hematopoietic precursor cells, expanding these populations, or by enhancing the recovery of those cells that survive chemotherapy. Protective effects for bone marrow precursor cells could include placing them in a protected portion of the cell cycle, inducing scavengers that might render the chemotherapeutic treatments less toxic, or up-regulating repair mechanisms. Factors that could enhance the recovery might include the capacity of rhIL-1α to accelerate stem cell cycling or to up-regulate CSF receptors, rendering them more responsive to the hematopoietic stimulatory signals induced by a lethal dose of SFUra. In this regard, the recovery of progenitor cells in the bone marrow 4 days after a sublethal dose of SFUra showed significantly augmented levels of CFU in groups that received IL-1 alone or in combination with G-CSF after SFUra treatment (13).

The fact that IL-1 pretreatment plays an important role in rescuing mice from myelotoxicity is corroborated also by the observation that this IL-1 pretreatment schedule was able to protect mice from lethal toxicity induced by carboplatin and BCNU. Carboplatin is a DDP analogue which is selected for its lack of nephrotoxicity (which is dose limiting for DDP) but which has myelotoxic dose-limiting effects in mice and humans (31, 32), while BCNU is a nitrosourea compound reported to be myelotoxic (37). Pretreatment of mice with rhIL-1α protected them from lethal doses of carboplatin and BCNU. Pretreatment with rhIL-1α did not, however, prevent acute death induced by treatment with DDP and DOX, known to be nephrotoxic and cardiotoxic, respectively. Previous studies have reported that a 7-day infusion of IL-1 was able to protect against some of the hematological effects induced by a lethal dose of DOX, but no data were presented on whether such effects correlated with enhanced survival of these mice (38), suggesting that even if the

Table 1 Induction of cytokines following the i.p. injection of rhIL-1α* or rmTNFα

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Cytokine injected</th>
<th>Hours after cytokine injection</th>
<th>Cytokine activity assayed</th>
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<td>rmTNFα (5,000 units)</td>
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* BALB/c mice received i.p. injections of Hanks' balanced salt solution, rhIL-1α, or rmTNFα at the doses indicated. Pooled serum from 5 mice/group was obtained at either 1 or 3 h after cytokine injection and assayed for various cytokines as described in "Materials and Methods."  

* HBSS, Hanks' balanced salt solution; ND, not determined.

Fig. 7. Effect of rmTNF-α and rhIL-6 pretreatment on acute SFUra-induced toxicity. A, BALB/c mice (10 mice/group) were treated i.p. at different times and doses with rhIL-1α or rmTNF-α before the i.v. administration of a lethal dose of 280 mg/kg SFUra. rhIL-1α (5 X 10⁴ units/mouse) was given on day -1 (○) or on days -7, -5, -3, or -1 (●), or rmTNF-α (5 X 10³ units/mouse) was given on days -7, -5, -3, -1 (▲); rhIL-1α (500 ng/mouse) was given for 7 days before SFUra treatment (■); control mice received H₂O plus 0.5 NMS (□). rmTNF-α at the dose used was not toxic by itself (data not shown). B, BALB/c mice (10 mice/group) were treated i.p. daily for 7 days prior to the administration of a lethal dose of 280 mg/kg SFUra with rhIL-6 at 5 μg/mouse (●) and 1 μg/mouse (△) twice a day; with rhIL-6 at 1 μg/mouse twice a day and rmTNF-α at 5 x 10³ units/mouse (▲) or rhIL-1α at 500 ng/mouse (■). Control mice received H₂O plus 0.5% NMS twice a day (△). 5FU, SFUra.
IL-1 treatment enhanced hematopoietic recovery, it might not be sufficient to enhance survival, based on an inability to protect against some nonhematological toxicity.

However, IL-1 pretreatment does seem able to protect against some nonhematological toxicities, since bone marrow transplant was not able to rescue mice that had received a lethal dose of Cy, but IL-1 pretreatment did protect. This might be due to the fact that Cy, although known to be myelosuppressive, might affect not only the hematopoietic precursor cells but also the hematopoietic microenvironment (stromal cells) so that the newly transplanted cells would not be able to home and/or proliferate, or that Cy may lethally affect other organs. Thus, rhIL-1α pretreatment might be able to protect the hematopoietic microenvironment, allowing a faster recovery after Cy treatment. IL-1 has been reported to stimulate in vitro fibroblasts and endothelial cells, which are important components of the bone marrow microenvironment (39). Although acute cardiopulmonary toxicity has been reported at very high doses (40), no other clear acute dose-limiting toxicities have been reported at this dose of Cy (390 mg/kg). In addition, it is known that Cy treatment is immunosuppressive (41) and that an increased resistance to infections occurs after IL-1 treatment (42, 43). Such effects of IL-1 may contribute to its protective action in mice treated with lethal doses of Cy which would correlate with reported faster hematological recovery in the IL-1-pretreated mice (44). The increase in natural resistance to infection has been attributed to the enhancement of humoral (45) and/or cellular defense mechanisms. With respect to humoral defense mechanisms, IL-1 is known to induce several hepatic acute phase proteins (metal ion transport proteins such as transferrin and ceruloplasmin and scavengers of free radicals such as metallothionein and ceruloplasmin), as well as other proteins directly or indirectly involved in host defense (C-reactive protein, haptoglobin, etc.) (46). With respect to cellular defenses, IL-1 treatment has been reported to affect both monocytes and T-cells (for a review, see Ref. 23).

The ability of IL-1 to stimulate various different host cell types makes it difficult to establish whether the observed chemoprotective effects of IL-1 are direct or are mediated through the induction of other multifunctional cytokines. IL-1 has been shown to induce a colony-stimulating activity in the serum of treated mice that peaks at 2 h after treatment (Table 1; Ref. 21), and this induced activity could be responsible in part for the effects of IL-1. Also, IL-6 and TNF activities were rapidly induced after an in vivo treatment with IL-1 (Table 1). IL-6 and TNF are multifunctional cytokines, based on their broad spectra of biological activities (14). TNF, which has been reported to have radioprotective effects (34), was unable to confer chemoprotection under any of the treatment schedules tested, and this might be consistent with reports suggesting that it can inhibit hematopoiesis (47, 48). IL-6 stimulates hematopoiesis and appears to play a key role in inflammation by inducing acute phase proteins (23). It has also recently been reported to have potent chemorefractory effects, since 7 days of continuous infusion was able to rescue mice that had been treated previously with a lethal dose of 5FUra (33). We noted that 7 days of rhIL-6 pretreatment was able to rescue, in a dose-dependent manner, mice that had been treated subsequently with an acutely toxic dose of 5FUra (33). Studies are planned to determine whether the in vivo treatment with antibodies directed against murine IL-6, as well as IL-1 type I and II receptors and various CSFs, would abrogate the IL-1-induced chemoprotective effects against 5FUra. The finding that IL-6 can at least partially substitute for IL-1 in chemoprotection may be of considerable practical importance, since it may be less toxic in humans.

T-cells are important in cellular host defense and are a major source of hematopoietic growth factors (49). Therefore, we tested the possibility that T-cells might play a major role in IL-1-induced chemoprotection. Based on the data obtained, it seems that T-cells may contribute to chemoprotection, since IL-1 pretreatment is only about half as effective in athymic mice as it is in syngeneic euthymic mice for protection from lethal doses of 5FUra. In this regard, Dubois (50) and colleagues have reported that an antibody to the type I IL-1 receptor found on T-cells inhibits most of the inducible IL-1 receptor expression on bone marrow cells. Since hematopoietic progenitors express only type II IL-1 receptors, this result suggests that these type II receptors are up-regulated indirectly through the type I receptors. Alternatively, it remains possible that the few T-cells normally present in nude mice could mediate the partial protective effects observed. However, the transfusion of T-cells into nude mice has to date failed to restore IL-1-induced chemoprotection to the levels achieved in syngeneic euthymic mice (data not shown), suggesting that cells other than T-cells, or other mechanism(s), must also be involved. The rationale for such a hypothesis is that T-cells express only the type I IL-1 receptor, while the type 2 receptor is found on monocytes, B-cells, and neutrophils (51). Thus, if the chemoprotective effects of IL-1 are indirect, they could be mediated through cells expressing either or both of the different receptors for IL-1. Thus future studies will focus on the ability of antibodies or antagonists (52) against the IL-1 receptor to inhibit chemoprotective effects. Similarly, since athymic mice contain considerable numbers of natural killer cells, studies will be performed to determine whether the depletion of natural killer cells completely abrogates the chemoprotective effects of IL-1 in athymic mice.

The data presented herein extend the premise that pretreatment with IL-1 may be a valid tool in cancer treatment, as part of a combined modality approach with agents whose acute dose-limiting toxicity is myelotoxicity. It must be emphasized, however, that protection from the acute dose-limiting effects of chemotherapy and/or radiotherapy may not extend to protection from later toxic effects, and additional dose-limiting toxicities may become evident upon dose escalation of chemotherapeutic drugs.

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CHEMOPROTECTIVE EFFECTS OF rhIL-1


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