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THE INFLUENCE OF IL-1 TREATMENT ON THE RECONSTITUTION OF THE HEMOPOIETIC AND IMMUNE SYSTEMS AFTER SUBLETHAL RADIATION

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The influence of IL-1 administration on the recovery of the hemopoietic and immune systems from sublethal irradiation was assessed. Mice were irradiated (750 R) and injected twice daily with purified recombinant derived IL-1β (200 ng/injection). At various times after irradiation, the functional capacity of the hemopoietic and immune systems was determined. It was found that IL-1 therapy resulted in a significantly greater number of granulocyte-macrophage-CSF responsive colony-forming cells in the bone marrow of the irradiated mice on days 5 and 11 postirradiation but not at later times. In addition the radiation induced neutropenia recovered quicker in the IL-1-treated mice with significantly greater numbers of peripheral blood granulocytes being seen on days 15 and 20 after irradiation. The influence of IL-1 therapy on the recovery of the immune system was also assessed. Of note was the observation that mice receiving IL-1 therapy had chronically hypoplastic thymi. Although thymic cellularity increased with time after irradiation in the control mice, there was no such increase in the IL-1-treated mice. Similarly, the number of pre-B cells in the marrow of these mice was also diminished. Thus, in the IL-1-treated mice the regeneration of the peripheral immune function was retarded, characterized by a general lymphopenia and decreased splenic responses to mitogenic stimuli.

Chemical- or radiation-induced cytoreduction is used therapeutically for the treatment of neoplasms and also for conditioning before bone marrow transplantation. This treatment invariably leads to a decrease in bone marrow function of variable duration which is reflected in a substantial decrease in mature blood elements especially granulocytes or PMN[2] (1, 2). Many of these treatment regimens especially whole body irradiation are also immunosuppressive (3). During this period of neutropenia and decreased immune function greater susceptibility to infection has been observed (4). In the majority of cases, marrow function, blood granulocyte numbers, and immune reactivity eventually recover, but the therapeutic efficacy of cytoreductive treatment tends to be limited by its bone marrow toxicity.

The production of mature blood cells from bone marrow-derived precursors and stem cells has long been recognized to be regulated by soluble cytokines known as CSF (5). These mediators stimulate the production of mature blood cells by inducing the proliferation, survival, and differentiation of immature progenitors. Although it is known that cytoreductive therapy decreases the number of stem cells, it is possible that the marrow dysplasia observed after cytoreductive therapy could be caused in part by the inadequate production of one or more of the CSF. The recent results with G-CSF to hasten hemologic recovery in cyclophosphamide-treated primates and 5-fluorouracil-treated mice indicates that after cytoreductive therapy CSF production may be suboptimal and that the hemopoietic stem cells will respond to exogenous cytokines (6, 7).

Recently the cytokine IL-1 was shown that in addition to its numerous other effects (reviewed in Ref. 8), it also has hemopoietic activity (9). IL-1 in of itself does not stimulate colony formation by bone marrow cells and thus cannot be classified as a CSF, however, it potentiates the effect of other CSF on bone marrow cells and causes increased production of CSF from cultured fibroblasts and endothelial cells (10, 11). In addition, the administration of IL-1 to normal mice is radioprotective and has been shown to increase the number of proliferating cells in the bone marrow (12, 13). Thus, IL-1 may be an ideal therapeutic agent in compromised bone marrow since it can stimulate the production of and potentiate the effects of many other CSF. Indeed this has recently been demonstrated by Moore and Warren (14) who found that IL-1 treatment augmented hemologic recovery in mice treated with 5-fluorouracil.

In this communication we use purified recombinant derived IL-1β as a therapeutic agent to ameliorate the decrease in colony-forming capacity of the bone marrow and resultant neutropenia after sublethal irradiation in mice. We find that twice daily administration of IL-1 after exposure to 750 R results in increased colony-forming capacity in the bone marrow and an accelerated recovery from neutropenia. However, the IL-1 treatment appeared to retard the recovery of the immune system because the thymus remained aplastic after irradiation and the responses of the spleen cells to T cell and B cell mitogens were decreased compared with untreated irradiated mice.

MATERIALS AND METHODS

Mice. C57BL/6J female mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The mice used in these experiments were between 13 and 16 wk of age and in general weighed between 19 and 22 g.

Irradiation. Mice were irradiated (750 R) in a rotating plastic holder. The ^{137}Cs source (J. L. Shepherd Co., Glendale, CA) delivered

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[2] Abbreviations used in this paper: PMN, polymorphonuclear leukocytes; GM-CSF, granulocyte-macrophage CSF; G-CSF, granulocyte CSF; MSA, mouse serum albumin; WBC, white blood cells.

a dose rate of 265 rad/min. The mice were irradiated six at a time on a rotating turntable.

Lymphokines. Human IL-1β was produced in *Escherichia coli* transfected with the published gene sequence (15). The IL-1β was purified to homogeneity as previously described (16). The IL-1β used in these experiments was stored in solution at 4°C at a concentration of 1 mg/ml. Immediately before injection it was diluted to the appropriate concentration in a solution of 1 μg/ml of MSA (Sigma Chemical Co., St. Louis, MO) in pyrogen-free saline. Control mice received an identical volume of the MSA solution The endotoxin level in the IL-1β stock and diluent was assessed by the *Limulus* amebocyte assay. The diluted material which was injected had endotoxin levels between 13.6 and 35.2 pg.

Purified recombinant derived murine GM-CSF was produced in house (Immunex Corp., Seattle, WA) as previously described (17) by using the published gene sequence (18). CSF-1 used in these experiments was derived from the conditioned media of the L929 cell line and partially purified by DEAE ion exchange chromatography. Purified recombinant derived G-CSF was also produced in house by using the published gene sequence (19) and expressed in yeast (17). The crude supernatant of the WEHI-3B cell line was used as a source of IL-3.

Assay of CFU. Cell suspensions were made from a tibia and femur from the experimental mice. The viable cells were counted in a hemocytometer after dilution in trypan blue. The cells were then diluted to the appropriate concentration in RPMI 1640 media supplemented with 10% FCS, 5×10^{-5} M 2-ME, sodium pyruvate, and nonessential amino acids. The frequency of CFU was assessed by the limiting dilution method described by Crapper and Schrader (20). Briefly, for colony growth, the medium was supplemented with 0.3% agar (Difco Laboratories, Detroit, MI). The various concentrations of cells were plated in 96-well flat bottom culture plates (Costar, Cambridge, MA) in a volume of 0.2 ml which contained 50 ng/ml of GM-CSF or G-CSF or 250 U/ml of IL-3. Twenty-four replicate cultures were established for each cell dose. For the irradiated mice, the cell concentrations used were 5,000, 1,000, 500, and 250 cells/well. The plates were incubated at 37°C in 7.5% CO_2 in a humidified incubator. After 8 or 9 days the number of wells which were positive for colony growth were enumerated through an inverted microscope. Colonies were scored positive if they had greater than 50 cells. The frequency of the CFU was calculated according to the maximum likelihood method as described by Taswell (21). The frequency was then multiplied by the number of bone marrow cells obtained from the mice which yields an absolute number of CFU per femur and tibia.

Preparation and analysis of cells. After anesthetization with ether, mice were bled via cardiac puncture. Sodium EDTA was used as an anticoagulant. WBC numbers were assessed by counting nuclei in a hemocytometer after dilution of the blood in Turks solution. Blood smears were made and stained with Wright's Giemsa stain. The slides were coded and the code broken only after microscopic evaluation of the WBC differential for the entire experiment. Peritoneal cells were obtained by lavage of the peritoneal cavity with 8 ml of HBSS with 2% FCS. The cell concentration was determined by counting an appropriately diluted aliquot in a hemocytometer. Differential analysis of the peritoneal cell population was done by microscopic analysis of Wright's Giemsa-stained cytospin preparations. A similar procedure was used to examine the cellular composition of the spleen and lymph nodes.

Response to mitogens. Spleen cells (2×10^5) were cultured in 96-well flat bottom plates in a 0.2-ml volume of RPMI 1640 containing 5% FCS, 5×10^{-5} M 2-ME, sodium pyruvate, and nonessential amino acids. The cultures were incubated in a humidified 7.5% CO_2/air mixture for 72 h. [^{3}H]TdR (0.5 μCi/well) was added for the last 6 h of cultures. The plates were harvested by using an automated multiple sample harvester and the [^{3}H]TdR incorporated was assessed by liquid scintillation spectrometry. Con A was used at a final concentration of 2.5 μg/ml. LPS was used at 50 μg/ml. GM-CSF was used at 50 ng/ml and concentrated partially purified L929 culture SN was used as a source of CSF-1 (1/500 final dilution).

Statistical analysis. The statistical significance of differences observed between the IL-1-treated and control mice was determined by using the two-tailed Student's *t*-test. Each experimental point described is the arithmetic mean of the values obtained from four individually assayed mice per group unless otherwise indicated. The data reported herein have been reproduced at least three times and representative results are shown.

Immunofluorescent staining and flow cytometry. Cells (10^6) were incubated at 4°C for 30 min with mAb in amounts which were predetermined to be saturating, washed in PBS with 0.1% BSA and 0.1% NaN_3, and incubated for 30 min at 4°C with fluorescein-conjugated anti-rat κ-chain (Becton Dickinson, Mountain View, CA). The culture supernatant of the 14.8 cell line was used to identify pre-B cells (22). Expression of the Mac-1 Ag was determined by staining with the culture supernatant of the M1/70 cell line (23).

Flow cytometry was performed on a FACS (EPICS-C, Coulter Corp., Hialeah, FL). Data were collected on 10^4 cells and were analyzed for minimum numbers of positive cells by determination of the percentage of positive cells above the fluorescent intensity of cells stained with the fluorescent reagent alone.

RESULTS

IL-1β therapy accelerates regeneration of myeloid cells after sublethal irradiation. The ability of IL-1β therapy to ameliorate the radiation-induced myeloid hypoplasia and neutropenia was tested by exposing mice to 750 R followed by twice daily i.p. injections of either IL-1β or MSA (200 ng/injection) for the duration of the experiment. Mice were sacrificed at 4- or 5-day intervals for 20 days and their myeloid compartment assessed by measuring the colony forming capacity of their bone marrow cells in response to GM-CSF as well as the number of PMN in the peripheral blood. The colony-forming capacity of the bone marrow from the irradiated mice in response to GM-CSF is shown in Figure 1. As can be seen, IL-1 therapy significantly increased the number of colonies on days 5 and 11. On day 5 the IL-1-treated mice had approximately eightfold greater CFU than did the control mice and on day 11 the difference was approximately fivefold. Thereafter the number of colonies obtained from the IL-1-treated group were essentially the same as the MSA-injected irradiated mice. The increase in the colony-forming capacity of the bone marrow was due to an increase in the precursor frequency of CFU. There was no significant difference in the number of bone marrow cells obtained from the IL-1-treated and control irradiated mice (data not shown). We have also assessed the possibility that MSA could inhibit hemopoietic recovery and have found no evidence for this (data not shown).

The increased colony-forming capacity of the bone

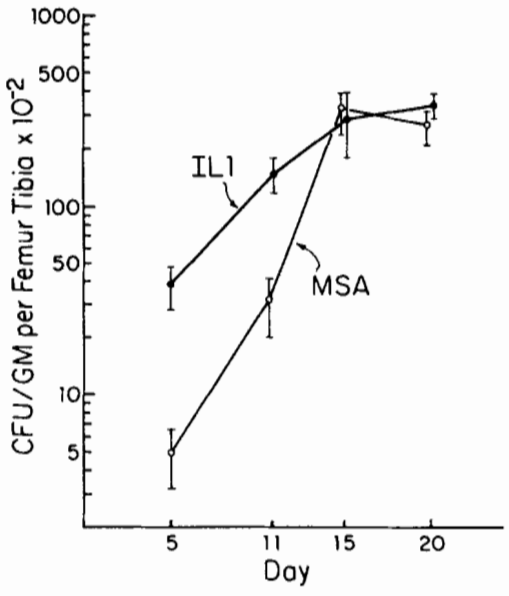

Figure 1. The effect of IL-1 therapy on the GM-CSF responsive CFU per tibia, femur in irradiated mice. The precursor frequency of the CFU of the bone marrow cells was assessed in a limiting dilution assay. The absolute number of CFU per femur, tibia was calculated by multiplying the yield of bone marrow cells by the precursor frequency. The results are the mean ± SEM of the values from four mice/group. The differences are statistically significant on days 5 and 11. Values from normal mice ranged between 42 and 79 × 10^3 GM-CSF responsive CFU per tibia, femur.

marrow from the IL-1-treated mice was also seen in response to other hemopoietic factors as well. In Figure 2, the colony-forming response of the bone marrow to G-CSF and IL-3 is also shown. Here it can be seen that on day 4 after irradiation the marrow from the IL-1 treated mice had a significantly greater colony-forming capacity in response to G-CSF and IL-3 as well as GM-CSF than did the MSA-treated mice. Again this increase reflected a greater precursor frequency and not an increase in marrow cellularity between the two groups. Thus, treatment with IL-1 results in an increased colony-forming response to all three factors 4 days after irradiation.

The efficacy of the IL-1 therapy on the regeneration of mature PMN was assessed by evaluating their number in the peripheral blood and peritoneal cavity. In Figure 3, it can be seen that the irradiation induces a severe neutropenia in both the IL-1-treated and control groups when assessed on day 5. At this time the number of PMN is decreased to between 2 and 4% of normal values. However, the IL-1 therapy group shows an accelerated regeneration of PMN with statistically significant differences being seen on days 15 and 20. In fact the PMN numbers in the IL-1 treated group are within the normal range on day 20. This increase in PMN numbers in the IL-1 therapy group is due to an increase in the proportion of PMN and not to an increase in the absolute number of WBC. There was no significant increase in the WBC count in the IL-1 therapy group over the control group at any of the time points measured (data not shown). Additionally, the increase in the PMN population in the IL-1 treated mice resulted in a decrease in the proportion of lymphocytic cells in the peripheral blood.

One unusual aspect of daily cytokine administration via i.p. injection is the tendency of the cytokines to exert a chemoattractant effect, thereby increasing the number of cells at the injection site (24). In Figure 4, the total cellularity and the number of PMN in the peritoneal cavity are shown. It can be seen that the mice given IL-1 therapy had a greater total peritoneal cellularity by almost 10-fold on day 15 and threefold on day 20 than MSA-treated mice. In addition, the number of PMN in the peritoneal cavity was also dramatically increased at both time points. On day 15, the IL-1-treated mice had approximately sevenfold more PMN and on day 20 the number of PMN in the peritoneal cavity of the IL-1-treated mice was 25-fold greater than the control mice. The remainder of the peritoneal cells in the IL-1-treated mice were predominately macrophages with a minor proportion being lymphocytes. Thus, daily IL-1 therapy after sublethal irradiation results in a more rapid recovery from myeloid hypoplasia reflected by increased levels of PMN in both the peripheral blood and peritoneal cavity.

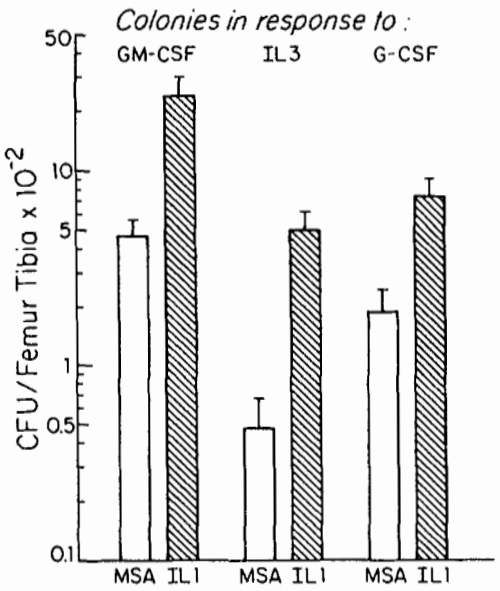

Figure 2. The effect of IL-1 therapy on GM-CSF, G-CSF, and IL-3 responsive CFU. On day 4 after 750 R the colony-forming capacity of the bone marrow cells in response to IL-3, G-CSF, and GM-CSF was assessed in limiting dilution assay. The obtained precursor frequency was multiplied by the number of bone marrow cells isolated from the femur and tibia of the mice to obtain the absolute number of CFU. The results are the mean ± SEM from four mice per group.

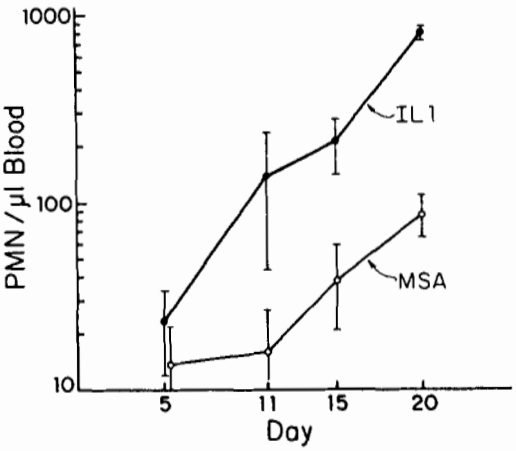

Figure 3. The effect of IL-1 therapy on peripheral blood PMN numbers after 750 R. The irradiated mice were injected twice daily with 200 ng of IL-1 or MSA until the day of death. The number of PMN/μl blood was determined by multiplying the percentage of PMN by the number of WBC. The values represent the mean (±SEM) of four individually assayed mice/group. The differences are statistically significant on days 15 and 20.

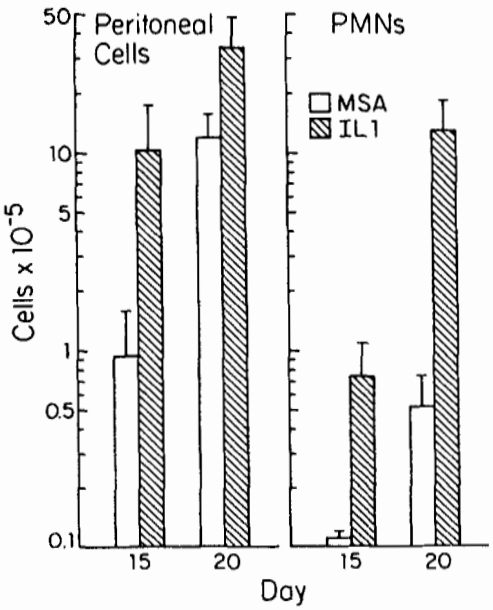

Figure 4. Effect of IL-1 therapy on peritoneal cavity cellularity after 750 R. Irradiated mice were injected twice daily with IL-1 or MSA (200 ng/injection). At days 15 and 20 after irradiation, the mice were killed and peritoneal cells obtained by lavage. The data are mean (±SEM) of four individually assayed mice. The differences between the total peritoneal cell yield on day 15 and the total number of PMN on day 15 and 20 are statistically significant ($p < 0.01$).

IL-1 therapy after sublethal irradiation retards the regeneration of immune reactivity. Exposure of mice to sublethal irradiation causes a severe depletion in the number of lymphocytes and markedly depresses immune reactivity (3). It was of interest to determine what effect daily IL-1 therapy had on the regeneration of the immune system.

First we assessed the proliferative response of the spleen cells from the mice to various mitogens 20 days after irradiation. As can be seen in Table I, the response of spleen cells from the IL-1-treated mice to the T and B cell mitogens, Con A and LPS, respectively, was less than that of the irradiated MSA-treated controls. In contrast, however, was the proliferative response of the spleen cells to the hemopoietic factors GM-CSF and CSF-1. Here the spleen cells from the IL-1-treated mice responded better than the MSA-treated controls. Thus, these results suggest that the continued administration of IL-1 causes the spleen to support mainly myeloid differentiation. This is supported by the cytologic analysis of the spleen cells. Microscopic evaluation of Wright's Giemsa-stained cytocentrifuge preparations revealed that the spleen cells from the IL-1 treated mice predominantly consist of myeloblasts and promyelocytes (Table II). In contrast the spleen cells from the MSA treated mice consist mainly of lymphocytes. Thus, at least in the spleen, the enhanced production of PMN by the IL-1-treated mice is at the expense of the lymphocytic population.

It is possible that treatment with IL-1 alters the homing patterns of the lymphocytes which would result in splenic lymphopenia. To examine this possibility, the absolute number of lymphocytes in the spleen, peritoneal cavity, peripheral blood and lymph nodes was assessed 20 days following irradiation. This analysis is shown in Table III. In each instance the number of lymphocytes in the IL-1-treated mice is less than that of the MSA-treated mice generally by twofold. Thus, prolonged IL-1 therapy results in a generalized deficit of lymphocytes at least in these tissues. Flow cytometric analysis of these populations with FITC-conjugated αThy-1 and $\alpha\mu$ reagents revealed that both the T cell and B cell components were diminished (data not shown) in the IL-1 treated mice. Thus, the deficit of lymphocytes is not attributable uniquely to a lack of T cells or B cells.

Since exposure to 750 R results in marked lymphocyte death (3), the cells to a large extent must be regenerated by differentiation from immature precursors. In order to assess the lymphopoietic compartments in the sublethally irradiated mice, we examined thymic cellularity as a measure of T cell production and the number of 14.8^+ cells in the bone marrow as a measure of the production of B cells.

In Figure 5, the recovery of thymic cellularity after 750 R is shown. Here it can be seen that the thymus in the MSA-treated mice increased in cellularity over the duration of the experiment. Surprisingly, however, in the IL-1-treated mice, the thymic cellularity did not increase but remained fairly constant at approximately 10×10^6 cells. Immunofluorescent staining and flow cytometric analysis of these thymocytes revealed a marked absence of the $L3T4^+/Lyt\text{-}2^+$ population.[3] Thus, the regimen of IL-1 administration although beneficial for myelopoiesis inhibits its thymic reconstitution. We explored the possibility that a lower dose of IL-1 would allow thymic recovery and still augment hemopoietic recovery. After 750 R mice were treated with different doses of IL-1 and the ability of IL-1 to increase GM-CSF responsive CFU on day 5 was compared with the thymic cellularity on day 14 (Fig. 6). Here it can be seen that IL-1 therapy even at 50 ng twice a day severely retarded the regeneration of thymic cellularity (Fig. 6*B*) although stimulating a suboptimal increase in CFU (Fig. 6*A*). These results suggest that there is not a dose of IL-1 which would allow thymic repopulation and optimally enhance the colony-forming capacity of the bone marrow.

The production of B cells was evaluated by quantitating the number of pre-B cells in the marrow of these mice by using immunofluorescent staining with the 14.8 mAb and flow cytometric analysis. The 14.8 mAb identifies a surface Ig-negative bone marrow population which has the capacity to differentiate into surface Ig-positive cells in vitro and in vivo (26). These results, shown in Table IV, clearly demonstrate that on day 20 after irradiation the number of pre-B cells in the marrow of the IL-1 treated mice is significantly reduced when compared with the irradiated MSA-treated mice, suggesting that the production of B cells is also dramatically impaired. Similar findings were obtained on day 14 (data not shown). In contrast there is an increased number of $Mac\text{-}1^+$ cells in the bone marrow of the IL-1-treated mice. The Mac-1 Ag

[3] **Morrissey**, P. J. *Submitted for publication.*

TABLE I

Proliferative response of spleen cells 20 days after exposure to 750 R[a]

Spleen Cells from Mice Treated with:	Response (Δ cpm $\times 10^{-3}$) to			
	Con A	LPS	GM-CSF	CSF-1
MSA	46.1 (6.3)	31.4 (7.9)	31.2 (5.1)	17.2 (4.6)
IL-1	7.9 (8.1)	10.1 (5.4)	66.5 (8.0)	32.3 (6.1)
Normal (unirradiated)	215.4	100.3	10.5	6.0

[a] The values are the arithmetic mean (SEM) of four individually assayed mice (except the normal control). The average [^{3}H]TdR uptake for cells cultured in the absence of mitogens were: 16,175 for the IL-1-treated mice, 9,063 for the MSA-treated mice and 4,351 for the unirradiated control.

TABLE II

Cell populations in the spleen 20 days after exposure to 750 R[a]

Treatment after 750 R	Splenic Cellularity ($\times 10^{-6}$)	Percent Cells				
		Lymphocytes	Monocytes	Promyelocytes/metamyelocytes	PMN	Nucleated RBC
MSA	86	58	10	19	6	6
IL-1	64	10	14	59	8	7
Unirradiated	110	75	8	6	1	10

[a] The values are the arithmetic mean of four individually assayed mice. The level of lymphocytes was significantly greater in the MSA-treated mice compared with the IL-1-treated group ($p < 0.01$). The number of pro/metamyelocytes was significantly higher in the IL-1-treated group ($p < 0.01$). Neither the splenic size nor any other parameter differed significantly.

TABLE III

Quantitation of the lymphocyte population 20 days after 750 R[a]

Irradiated Mice Treated with	No. of Lymphocytes (cells × 10^{-6}) in:			
	Spleen	Peritoneal cavity	Blood	Lymph node
MSA	49.8	0.22	0.145	6.0
IL-1	6.4	0.11	0.074	3.4

[a] The number of lymphocytes was determined by multiplying the percentage of cells as lymphocytes (from Wright's Giemsa-stained slides) by the total number of cells obtained. For the estimation of the number of cells in the blood the total blood volume was assumed to be 5% of the body weight (25). The axillary, inguinal, and mesenteric lymph nodes were dissected and pooled for the estimation of their cellularity.

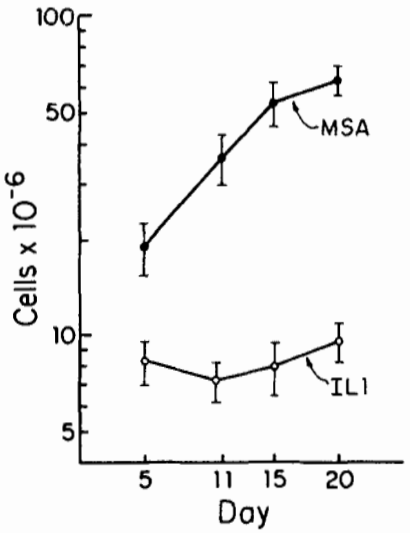

Figure 5. Effect of IL-1 therapy after 750 R on the regeneration of thymic cellularity. Irradiated mice were injected twice daily with IL-1 or MSA (200 ng/injection). At the indicated time, the mice were killed, the thymus dissected and teased into a cell suspension, and the cells counted. The data are the mean (±SEM) of four individually assayed mice. The difference in cellularity is statistically significant on days 11, 15, and 20 ($p < 0.01$).

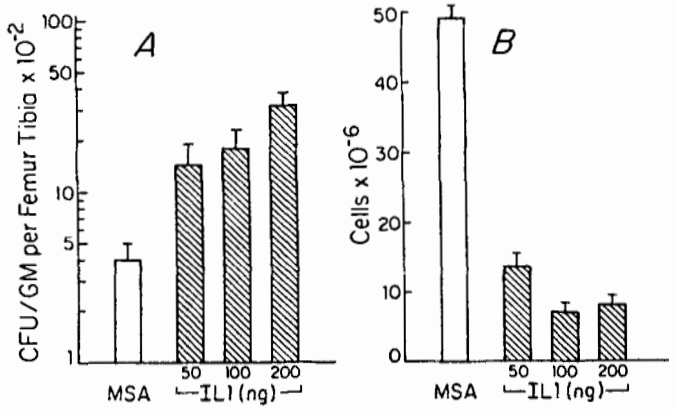

Figure 6. Effects of various doses of IL-1 on GM-CSF responsive CFU and thymic cellularity. Irradiated mice (750 R) were injected twice daily with the indicated amount of IL-1 or 200 ng/injection of MSA. Bone marrow CFU was assessed on day 5 and the thymic cellularity was assessed on day 14 as described.

has been shown to be expressed on macrophage/granulocyte progenitors as well as mature macrophages (23). These findings indicate that the marrow and spleen in the IL-1 treated mice are largely committed to the production of granulocytes and macrophages and that this may be to the detriment of B cell production. Thus, these data suggest that the deficit of lymphocytes seen in the IL-1 treated mice is due to decreased production.

TABLE IV

Proportions of 14.8⁺ and Mac-1⁺ cells in the bone marrow 20 days after irradiation[a]

Source of Bone Marrow Cells	Cellularity (× 10^{-6})	Percent Cells Staining Positively for:	
		Mac-1	14.8
Normal	27.5	38	19.8
750 R + MSA	20.3 (1.44)	15 (2.2)	28 (2.7)
750 R + IL-1	17.5 (2.9)	62 (4.8)	3 (1.9)

[a] The data are mean (SEM) of four individually assayed mice except the normal population which represents a single mouse. The bone marrow cells were isolated from a tibia and femur from each mouse. The differences between the MSA- and IL-1-treated groups are significantly different for both Mac-1 and 14.8 markers ($p < 0.01$). The data obtained from the normal mouse is representative of the values from unmanipulated mice.

DISCUSSION

In this report, we evaluate the efficacy of using the cytokine IL-1 to hasten the recovery of the hemopoietic and immune systems of mice exposed to 750 R. After sublethal irradiation, mice were injected i.p. with IL-1 or control carrier (MSA) and received 200 ng twice a day until sacrifice. We found that the IL-1 therapy did increase the number of bone marrow CFU formed in response to GM-CSF as well as G-CSF and IL-3. This increase was greatest at the earlier time points demonstrating that IL-1 hastened the recovery of the bone marrow CFU.

Assessment of PMN in the peripheral blood showed that their number was severely decreased by the irradiation to roughly 2% of usual. The administration of IL-1 did not prevent this neutropenia, however, IL-1 therapy resulted in a rapid increase in PMN with normal levels being reached by day 20. At this time the PMN number in the MSA treated mice reached only about 20% of normal levels.

Because the administration of cytokines intraperitoneally causes an increased accumulation of cells there, we examined the peritoneal lavage of the mice for total cell number and the cellular composition of the infiltrate. Here there was a remarkable increase in the cellularity of the peritoneal cavity in the IL-1 treated mice. These mice had significantly greater numbers of PMN in their peritoneal cavity than did the irradiated control mice. Thus, these data indicate that the production of PMN in the IL-1-treated mice is actually much greater than what is reflected in the assessment of the peripheral blood alone. One might expect that, if it were practical to administer IL-1 i.v. to mice, the levels of circulating PMN would rise more rapidly and dramatically. These data therefore indicate that daily IL-1 administration hastens the recovery of the myeloid compartment after sublethal irradiation.

The mechanism by which IL-1 exerts this effect is not addressed here but the findings of others suggest that it is mediated at least by stimulating increased production of CSF by fibroblasts or endothelial cells (10, 11). It is also possible that IL-1 could act directly on the hemopoietic cells thereby enhancing their response to CSF. Additionally, the ability of IL-1 administration to enhance myelopoiesis after irradiation is not unique to this cytoreductive modality because IL-1 therapy has recently been shown to hasten myeloid recovery after 5-fluorouracil administration in mice (14).

The effect of IL-1 administration on the recovery of the

immune system was seemingly to retard its regeneration. Exposure of mice to 750 R results in a severe depletion (by 90%) of lymphocytes in the lymph nodes, spleen, and thymus (P. J. Morrissey, personal observations). Treatment of the irradiated mice with IL-1 although augmenting myelopoiesis tended to delay the recovery of the immune system. Overall the IL-1-treated mice showed an increase in the number of lymphocytes over the length of the experiment; however, the increase was less than that observed in the control irradiated mice.

Most dramatic of these observations was the chronic hypocellularity of the thymus. After irradiation, thymic cellularity increased with time in the control, MSA-treated mice. However, the cellularity of the thymus in the IL-1-treated mice did not increase significantly above that observed immediately postirradiation for the duration experiment. Additionally, examination of the bone marrow at this time revealed a decrease in the number of cells expressing the 14.8 Ag (a marker expressed by cells committed to B lymphocyte development) and an increase in the number of Mac-1^+ cells.

The recovery of the peripheral immune system also seemed retarded by the continued administration of IL-1 therapy. Analysis of the cellular composition of the spleen showed that in the IL-1-treated mice, it was still largely a hemopoietic organ. This was reflected by the response of the spleen cells to the mitogenic stimuli. The spleen cells from IL-1-treated mice responded poorly to Con A and LPS but had a better response to the hemopoietic factors GM-CSF and CSF-1. Cytologic analysis of the spleen revealed a decreased number of lymphocytes. The lack of lymphocytes did not appear to be due to altered homing since assessment of the number of lymphocytes in the blood, lymph nodes, peritoneal cavity and spleen demonstrated that the IL-1-treated mice had an absolute deficit of lymphocytes.

The mechanism by which immune regeneration is inhibited is not yet known. It is possible that the long term administration of IL-1 results in the stem cell pool being overly committed to myelopoiesis at the expense of lymphopoiesis. This hypothesis is supported by the observation that the bone marrow of the IL-1-treated mice had increased numbers of Mac-1^+ cells but fewer 14.8^+ cells than the irradiated control mice.

This explanation may not account for the thymic hypocellularity, however. It is generally agreed that there are very few thymocyte precursors in the bone marrow and that very few precursors are required to repopulate the thymus (27, 28). Thus, it is difficult to envision that such a total commitment to myelopoiesis exists that would prevent the necessary few pre-thymocytes from repopulating the thymus. In addition, a radiation-resistant intrathymic stem cell has been described with the capacity to repopulate the thymus after irradiation (29). Therefore, the inhibition of thymic regeneration is probably due to a mechanism other than the increased rate of myelopoiesis.

Recently, evidence has been produced that in vivo IL-1 administration results in increased ACTH elaboration and consequently increased production of corticosteroids by the adrenal cortex (30–32). Corticosteroids are well recognized for their ability to induce thymic hypoplasia (33). Thus, the observed lack of thymic regeneration in the irradiated IL-1-treated mice could be due to the effects of increased levels of corticosteroids on thymic cellularity. Indeed, we have shown that injection of normal mice with 200 ng of IL-1 twice daily for four days results in a 90% decrease in thymic cellularity and increased serum levels of corticosteroids (see footnote 3). Thus, injection of this dose of IL-1 can produce steroid levels sufficient to effect thymic cellularity. It is not clear if these levels of corticosteroids produced in response to IL-1 can effect the number or function of lymphocytes residing in the spleen or lymph nodes. We are currently investigating this question.

A well recognized complication of clinical bone marrow transplantation is the prolonged time required for immune function to reappear (34). It is interesting to speculate whether this may be due inpart to increased production of IL-1 as a consequence of infection or graft vs host disease. Thus, as seen in this system, chronically increased levels of IL-1 may be involved in the delayed recovery of immune function in these patients. There is little information concerning serum levels of IL-1 in bone marrow transplant patients however, and the observation that membrane bound IL-1 has biologically activity (35) suggests that there could be increased production of IL-1 in the marrow microenvironment which might not result in increased serum levels.

Overall, these results demonstrate that IL-1 is a useful therapeutic for stimulating myeloid recovery following the cytoreductive effects of irradiation. However, it appears that prolonged administration of IL-1 has a deleterious effect on the regeneration of the immune system. This suggests that IL-1 therapy may be most useful when given for a limited time immediately after radiation.

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