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Interleukin-1 is identical to hemopoietin-1: Studies on its therapeutic effects on myelopoiesis and lymphopoiesis

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Abstract

Conditioned medium from the human tumor cell line HBT 5637 possesses a unique hematopoietic activity, originally termed hemopoietin-1. Hemopoietin-1 alone does not stimulate bone marrow colony formation or proliferative responses *in vitro*, but rather potentiates responses to other hematopoietic growth factors, such as CSF-1 and GM-CSF. In studies designed to characterize the molecular nature of this factor, it was found by molecular, biochemical biological and serological criteria that all the hemopoietin-1 like activity could be attributed to IL-1α. The therapeutic potential of IL-1 was then tested in a system where myelopoiesis is depressed by whole body irradiation. After 750 R irradiation, mice were administered IL-1 twice daily for the duration of the experiment. Mice which received IL-1 treatment had an accelerated recovery of marrow colony forming capacity which was also reflected by significantly higher blood neutrophil levels as compared to control irradiated mice. IL-1 treated irradiated mice also had a significant increase in resistance to bacterial challenge 14 days post irradiation. Thus, IL-1 treatment was effective in augmenting myelopoiesis following sublethal whole body irradiation. The effects of the IL-1 treatment on the recovery of lymphocyte numbers was also assessed. Here the IL-1 treated irradiated mice had fewer lymphocytes and depressed mitogen responses by spleen cells. Indeed the thymus of the IL-1 treated irradiated mice remained chronically hypoplastic for the duration of the experiment. Although IL-1 treatment increased myeloid progenitors in the bone marrow, it caused a decrease in the frequency of pre-B cells. Thus, IL-1 administration is an effective treatment for accelerating myeloid recovery following the cytoreductive effects of irradiation, but the myelopoietic augmentation may be at the expense of lymphoid recovery.

Introduction

The growth and differentiation of myeloid cells is to a large part governed by a class of glycoproteins known as colony-stimulating factors (CSF). Three distinct colony stimulating factors (G-CSF, CSF-1 and GM-CSF) have been identified based on their ability to support the growth of granulocyte, macrophage or mixed granulocyte/macrophage colonies *in vitro* [1].

A number of years ago, a hematopoietic activity, produced by six day human placental cultures, and found to possess unique biological properties was described by investigators in the laboratories of Bradley and Stanley [2–4]. Stanley's group went on to determine that the human bladder tumor cell line HBT 5637 was a potent source of this novel activity which they designated hemopoietin-1. Hemopoietin-1 alone, neither stimulated the proliferation nor differentiation of

precursor colony forming cells. However, in the presence of a colony stimulating factor such as CSF-1, hemopoietin-1 could stimulate a normally non-responsive progenitor population to up-regulate CSF-1 receptor expression and to form colonies of high proliferative potential. Given that the bone marrow responder population was the result of 5-fluorouracil (5-FUra) treatment, and that many of the surviving responsive cells had high proliferative capacity, as measured by their ability to form colonies of greater than 0.5 mm in diameter [5], it was thought that hemopoietin-1 acted to drive the differentiation of progenitor hemopoietic cells into a CSF responsive state. It was unclear whether hemopoietin-1 represented a member of a class of regulatory factors which acted in a lineage restricted manner, or whether hemopoietin-1 was stage specific for all hemopoietic cells of an early differentiation state.

The existence of a factor which could drive early cells to become responsive to CSF was of great interest from the perspective of its potential therapeutic use. For instance, in clinical situations where the more mature hematopoietic cells had been depleted through cytoreductive modalities (e.g. radiation and chemotherapy), a factor which could accelerate the maturation of early cells could be of significant value in hastening the reconstitution of the myeloid compartment.

Here we review the evidence that hemopoietin-1 is actually identical to interleukin-1 (IL-1) and show that it has the capacity to potentiate colony forming responses as well as proliferative responses to CSF-1 and GM-CSF [6]. We have also used IL-1 in an *in vivo* sublethal irradiation model system and have found that it was effective in accelerating the recovery of myelopoiesis following sublethal radiation [7]. Thus, among its numerous known activities, IL-1 is also involved in the regulation of hematopoiesis which broadens the spectrum of its possible clinical uses.

Results

Development of a biological assay for a potentiating activity

We initially developed an assay designed to detect a factor capable of potentiating proliferative responses to GM-CSF. It was anticipated that this approach might permit us to characterize a novel GM-CSF specific potentiating activity, rather than the hemopoietin-1 activity being characterized by Stanley's laboratory. A number of sources were tested for this putative GM-CSF potentiating activity.

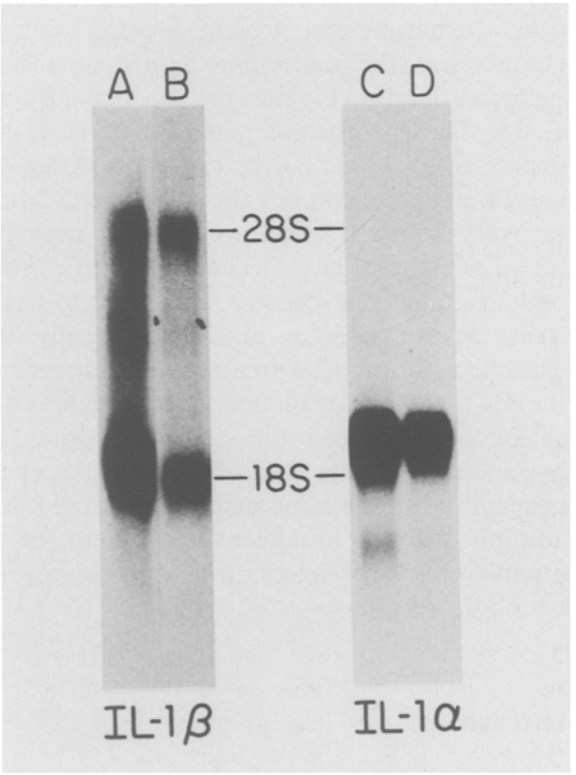

Fig. 1. RNA blot analysis of HBT 5637 and lipopolysaccharide-stimulated peripheral blood macrophage RNA. Two μg of HBT 5637 (lanes B and D) and peripheral blood macrophage (lanes A and C) poly(A)$^+$ RNA was run on duplicate blots and probed for IL-1β (lanes A and B) or IL-1α (lanes C and D). The positions of 18S and 28S rRNA were determined by direct visualization of the unbaked blot with a shortwave UV lamp. RNA was obtained from HBT 5637 cultures 48 hr after serum stimulation and from adherent macrophage cultures prepared and stimulated with 10 μg of lipopolyssaccharide per ml for 12 hr.

The conditioned media from stimulated human placenta, murine L929 cells and HBT 5637 cells were all found to be sources of a GM-CSF potentiating activity. However, the best source proved to be the six day serum free constitutive supernatants from HBT 5637 cells. Once the HBT 5637 cells had been selected as the source of the potentiating activity, efforts were directed toward confirming that this activity was mediated by a novel factor. The HBT 5637 conditioned media as well as poly A^+ messenger RNA extracted from the cells were evaluated for the presence of known factors. The cells were found to make abundant quantities of G-CSF mRNA. Unexpectedly, as can be seen in Figure 1, the cells were also found to produce abundant amounts of IL-1α and IL-1β mRNA (lanes B and D) at levels comparable to LPS stimulated murine macrophages (lanes A and C). This finding lead to the possibility that IL-1 could be involved in the hematopoietic activity found in the HBT 5637 conditioned medium.

Table 1. Potentiating activity of IL-1α, IL-1β, and HBT 5637 conditioned medium on bone marrow cells from 5-FUra treated mice.

Sample	GM-CSF	FUraBM assay, bioactivity units/ml*
Control	+	<1
IL-1α	−	<1
IL-1α	+	4.5×10^5
IL-1β	−	<1
IL-1β	+	3.1×10^4
Crude HBT 5637 CM	−	22
Crude HBT 5637 CM	+	1518
Purified HBT 5637 CM	−	31
Purified HBT 5637 CM	+	2100

rIL-1α and rIL-1β were used at 10 μg/ml. HBT 5637 crude conditioned medium was concentrated 10 ×. This material was purified by salting out chromatography, and the granulocyte CSF was removed by phenyl-Sepharose chromatography. rGM-CSF was added where indicated at 0.5 ng/ml.
* One unit of activity is defined as the amount of sample that induced 50% of the maximal response.

IL-1 possesses hematopoietic potentiating activity similar to hemopoietin-1

Based on the finding that HBT 5637 cells express IL-1 mRNA, and CSF potentiating activity of purified human recombinant IL-1 and HBT 5637 conditioned medium was assessed on bone marrow cells from mice which had been injected iv 24 hr previously with 5-FUra at a dose of 150 mg/kg. Treatment with 5-FUra selectively depletes the more mature cycling bone marrow cells while sparing the less mature non-cycling stem cells. Marrow from mice treated with 5-FUra is markedly hypocellular and has severely diminished responses to CSF *in vitro*. The CSF potentiating activity was measured by the augmentation of the response of depleted bone marrow cells to GM-CSF and CSF-1 both by proliferation and colony formation in soft agar. For example, as seen in Table 1, bone marrow cells from mice treated with the antimetabolite 5-FUra are severely depleted in their capacity to proliferate in response to GM-CSF. This response is significantly augmented by the addition of IL-1α, IL-1β, or the conditioned medium from HBT 5637 cells. Neither IL-1α nor IL-1β alone stimulates a response from the bone marrow cells. Similarly, HBT 5637 conditioned medium alone had a minimal effect. The low level of response by the bone marrow cells to the HBT 5637 conditioned medium is likely due to the presence of levels of CSF such as G-CSF [8]. To confirm that the potentiating activity of IL-1 and HBT 5637 conditioned medium was indeed influencing a hematopoietic response, the factors were tested for their ability to augment the colony forming response of bone marrow to GM-CSF and CSF-1. These data shown in Figure 2, demonstrate that the addition of IL-1 or HBT 5637 conditioned medium to colony forming assays using bone marrow cells from 5-FUra treated mice clearly augmented the response above that seen with GM-CSF

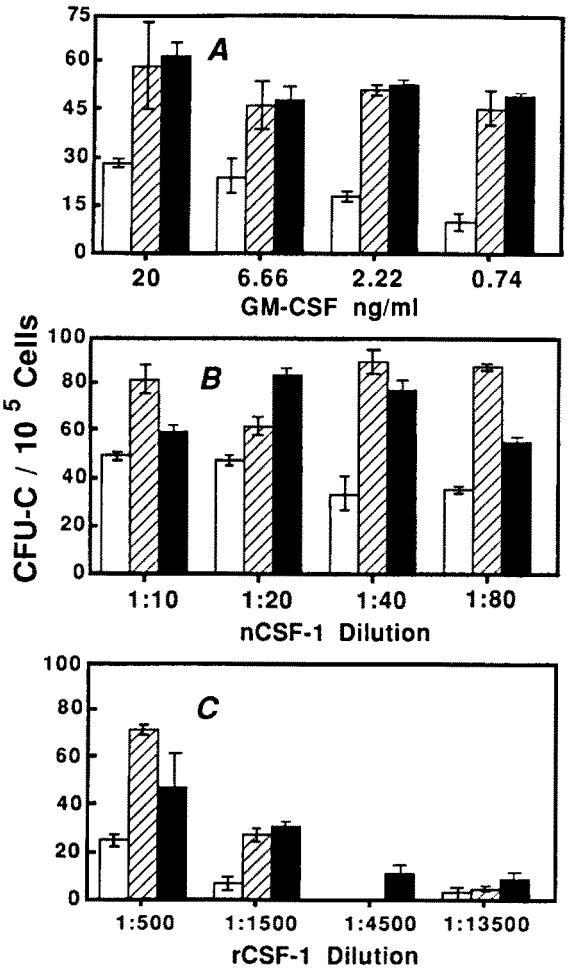

Fig. 2. Colony-forming unit (CFU-C) assay. The colony-forming ability of marrow from C3H/HeJ mice previously injected i.v. with 5-FUra in response to factor (open bars), factor plus IL-1α (hatched bars), or factor plus HBT 5637-conditioned medium (CM) (solid bars). The factors used were GM-CSF (panel A), natural purified CSF-1 (panel B) and recombinant CSF-1 (panel C). IL-1α was used at a concentration of 33 ng/ml. The source of nCSF-1 was L929 CM partially purified by DEAE ion-exchange chromatography to a concentration of ≈4 μg/ml. rCSF-1 was from yeast crude CM that had a rCSF-1 concentration of 1 μg/ml. Control yeast crude CM possessed no colony-stimulating colony-stimulating activity. Data represent the mean of triplicate cultures ± SD.

alone (Fig. 2A) or with natural purified CSF-1 or recombinant CSF-1 (Figs. 2B and 2C). The degree of augmentation was greater at the lower concentrations of GM-CSF and CSF-1. Thus, IL-1 and HBT 5637 conditioned medium have similar potentiating activities on both the proliferative and colony forming response of bone marrow cells from 5-FUra treated mice.

The hematopoietic activity of HBT 5637 conditioned medium is due to IL-1

Although IL-1 has similar hematopoietic potentiating activity as does HBT 5637 conditioned medium, it was still possible that another non-IL-1 factor was produced by these cells which also possessed this potentiating activity. Thus, experiments were designed to assess the contribution of IL-1 to the activity seen in the HBT 5637 conditioned medium. First the HBT 5637 conditioned medium was tested for activity in the bioassay for IL-1 to confirm that the IL-1 message was translated into biologically active protein. These data shown in Table 2, clearly demonstrate that the cells produced IL-1 activity as measured by the ability to induce IL-2 production from the LBRM-33-1A5 cell line [9]. This assay is generally 1000 times more sensitive than the thymocyte mitogenesis assay for IL-1 activity [9]. Thus, the HBT 5637 cell line expresses the gene for, and secretes biologically active IL-1.

The contribution of IL-1 to the potentiating activity of the HBT 5637 conditioned medium was assessed by determining if the two activities co-purified. Concentrated HBT

Table 2. Conditioned medium from the HBT 5637 cell line has IL-1 activity.

Sample	IL-1 Activity (units/ml)
IL-1α	1.53×10^9
IL-1β	2.63×10^8
Crude HBT 5637 CM	2.6×10^4
Purified HBT 5637 CM	4.5×10^4

rIL-1α and IL-1β were used at a concentration of 1 μg/ml. Crude HBT 5637 conditioned medium was concentrated 10 × as described in Table 1. IL-1 bioactivity was assessed by the induction of IL-2 production by the LBRM-33-1A5 cell line. The IL-2 activity was then quantitated by its ability to stimulate proliferation of CTLL cells.

5637 conditioned medium was subjected to ammonium sulfate salting out chromatography, phenyl-Sepharose chromatography and buffer electro-focusing. These procedures separate proteins by differential solubility, hydrophobicity and charge, respectively. In each case, the potentiating activity and IL-1 activity co-purified (data not shown) [6]. Thus, none of the chromatographic procedures were able to separate the potentiating activity from the IL-1 activity. It was possible, however, that the IL-1 bioassay was measuring a second, non-IL-1 activity. Therefore, a monoclonal antibody to IL-1α was used in an attempt to neutralize the activity of the HBT 5637 conditioned medium. This antibody, 2F4, is of mouse origin and recognizes human IL-1α. The conditioned medium from HBT 5637 cells was subjected to immunoprecipitation with the 2F4 mAb and then tested in both the IL-1 bioassay and the hematopoietic potentiating assay. As can be seen in Table 3, mAb 2F4 precipitated the bioactivity of both IL-1α and HBT 5637. The IgG1 isotype control did not precipitate a significant amount of the HBT 5637 potentiating activity. The antibody alone was neither stimulatory nor inhibitory in either assay (data not shown). Thus, these data, taken in total, provide strong evidence that the hematopoietic potentiating activity present in the HBT 5637 conditioned medium is due to IL-1α. In addition, it is shown that purified, recombinant IL-1β also possesses the potentiating activity, but is not responsible for the activity contained in the HBT 5637 conditioned medium either due to low levels of secretion or incorrect post-translation processing of the IL-1β message which is produced by the cells in large quantities.

IL-1 administration accelerates the recovery of the hematopoietic system following sublethal irradiation

There are clinical situations in which myelopoiesis is severely depressed as a result of radiation and chemotherapy [10, 11]. Although the myeloid compartment recovers in the majority of patients, a small percentage develop myeloid dysplasia. In these cases, the patients are unusually susceptible to infections while myeloid production is depressed [12]. These clinical situations could conceivably be ameliorated by treatment with factors which augment hematopoiesis, such as the CSF and/or IL-1. Therefore, we tested the efficacy of IL-1 administration on the recovery of the myeloid compartment following sublethal irradiation.

In this system, C57BL/6J adult female mice were exposed to 750 R from a ^{137}Cs source. It

Table 3. Immunoprecipitation of potentiating activity with IL-1α mAb 2F4.

Sample	mAb (10 μg/ml)	% Bioactivity Precipitated*	
		FUraBM assay	IL-1 thymocyte assay
IL-1α (1 μg/ml)	2F4	98	96
IL-1α (0.1 μg/ml)	2F4	100	99
IL-1β (1 μg/ml)	2F4	5	0
HBT 5637 PA	Isotype control	5	0
HBT 5637 PA	2F4	100	100

The IL-1α and IL-1β used were purified recombinant proteins. The HBT 5637 potentiating activity (PA) was partially purified by salting out and phenyl-Sepharose chromatography. The 2F4 mAb, specific for IL-1α is an IgG_1. The isotype control, AY-C12 is specific for an irrevelant antigen.

* IL-1 was measured in a thymocyte mitogenesis assay. The PA was monitored by the FUraBM assay. Specific activity of IL-1α was 1.5×10^5 IL-1 units/μg and 3.3×10^4 FUraBM assay units/μg. IL-1β had a specific activity of 2.6×10^4 IL-1 units/μg and 6.6×10^3 FUraBM assay units/μg. The HBT 5637 sample contained 3.4×10^3 IL-1 units/ml and 420 FUraBM assay units/ml.

should be noted that the mice received no exogenous bone marrow. The day following irradiation, purified recombinant IL-1 was administered ip, twice daily, at a dose of 200 ng/injection. IL-1 administration continued for the length of the experiment. At defined intervals, mice were sacrificed and the status of their myeloid compartment was assessed by studying both the bone marrow colony forming capacity in response to CSF and blood neutrophil levels.

In Figure 3, the colony forming capacity of bone marrow cells from irradiated mice treated with IL-1 and irradiated control mice is shown. It can be seen that the bone marrow cells from irradiated mice which received IL-1 had a significantly greater colony forming response to GM-CSF *in vitro* than did the irradiated controls on Days 5 and 11 post irradiation. Thereafter there was no significant difference. The increased colony forming capacity of the bone marrow cells from irradiated mice treated with IL-1 was also seen in response to IL-3 and G-CSF as well (data not shown) and again this increase was most significant at the early time points after irradiation [7]. Thus, administration of IL-1 following sublethal irradiation causes a significant increase in the recovery of the colony forming capacity of the bone marrow.

Next, the influence of the IL-1 treatment on the production of mature peripheral blood neutrophils was assessed. In Figure 4, it can be seen that the irradiation induces a severe neutropenia in both the IL-1 treated and control mice when assessed on Day 5. At this time the number of mature neutrophils is decreased by at least 95% of the numbers in normal mice. The mice treated with IL-1, however, recovered quicker from the nadir than did the control irradiated mice. The numbers of neutrophils in the peripheral

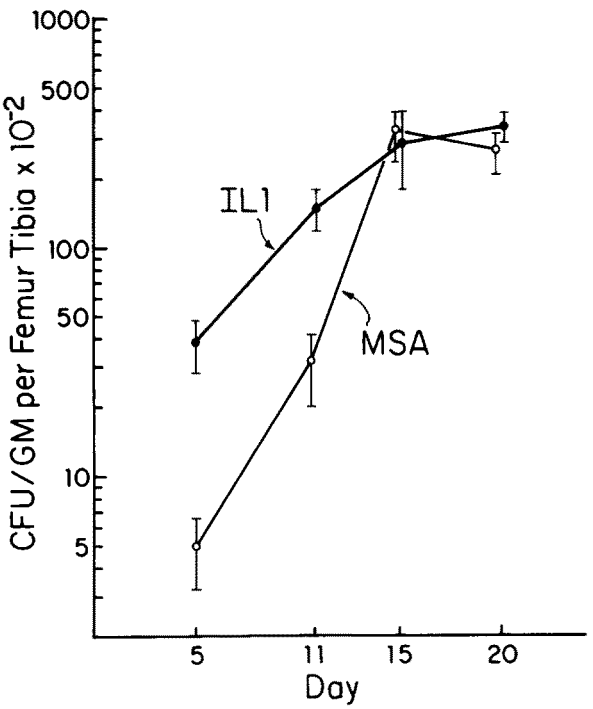

Fig. 3. The effect of IL-1 therapy on the GM-CSF responsive CFU per tibia, femur in irradiated mice. The precursor frequency of the CFU of the bone marrow cells was assessed in a limiting dilution assay. The absolute number of CFU per femur, tibia was calculated by multiplying the yield of bone marrow 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.

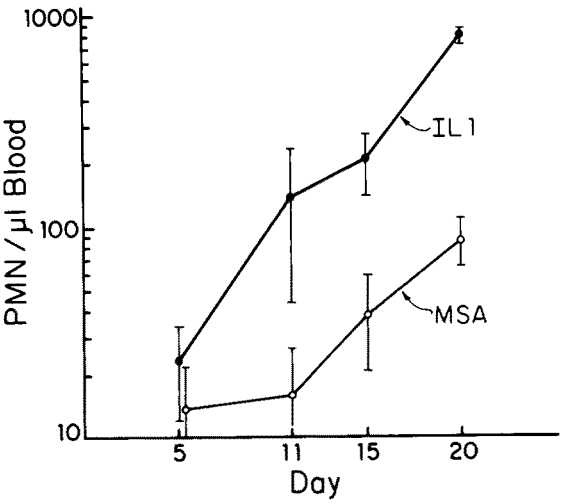

Fig. 4. The effect of IL-1 therapy on peripheral blood PMN numbers after 750 R. The irradiated mice were injected twice daily with 200 ng of IL-1 or MSA until the day of death. The number of PMN/µl blood was determined by multiplying the percentage of PMN by the number of WBC. The values represent the mean (± SEM) for four individually assayed mice/group. The differences are statistically significant on days 15 and 20.

blood was significantly greater than those in the irradiated control mice at Days 15 and 20. Indeed, the numbers are in the range of normal values on Day 20 and they are almost 10 fold greater than the value in the irradiated control mice.

In order to better assess the functional capacity of the myeloid compartment following irradiation, the IL-1 treated and control irradiated mice were challenged with a small number (100 organisms) of *S. typhimurium* bacteria on Day 14 post irradiation. Resistance to this bacteria is mediated primarily by macrophages [13]. As can be seen in Figure 5, the survival of the irradiated IL-1 treated mice is much greater than the MSA treated irradiated control mice. Although all the mice eventually succumb to the infection, the median survival time for the IL-1 treated mice was significantly greater (12.6 days for the IL-1 treated mice and 4.5 days for the control mice). Thus, IL-1 treatment not only hastens the recovery of neutrophil numbers following irradiation but enhances the resistance to an infectious agent.

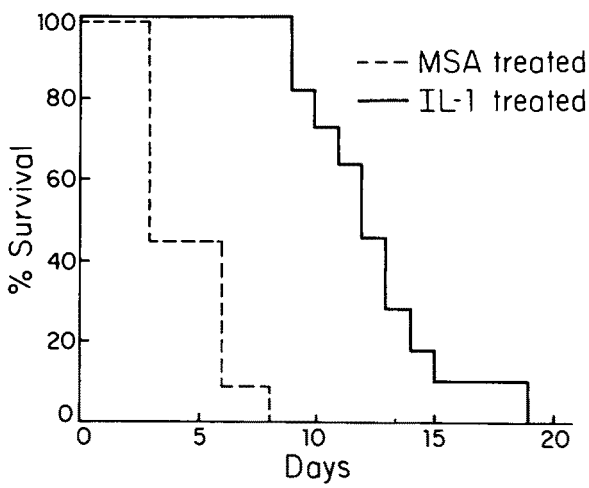

Fig. 5. IL-1 therapy increases the resistance of irradiated mice to a lethal dose of *Salmonella typhimurium*. A/J mice were irradiated (750 R) and injected twice daily with IL-1β or MSA. On the fourteenth day following irradiation, the mice were injected with 100 *S. typhimurium* bacteria i.p. 100% of the irradiated mice (IL-1 or MSA treated) which were not injected with *S. typhimurium* survived.

Recovery of the immune system from sublethal irradiation is retarded by IL-1 treatment

It is well established that radiation treatment is highly immunosuppressive [14]. Exposure of mice to 750 R results in over a 95% decrease in B cell and T cell numbers. As IL-1 has been described to be involved in the growth of thymocytes, T cells and B cells [15], it was of interest to determine what effect IL-1 administration had on the regeneration of the immune system following sublethal irradiation. Thus, twenty days after irradiation, IL-1 treated and control mice were examined for immune function and number of lymphocytes. The response of the spleen cells from these mice to the mitogens Con A and LPS are shown in Table 4. Here it can be seen that the response of the spleen cells from the IL-1 treated mice were less than that seen in the control irradiated mice to both Con A and LPS. Also the spleen cells were tested for their proliferative response to the hematopoietic factors, GM-CSF and CSF-1. In this situation the spleen cells from the IL-1 treated irradiated mice were in fact significantly greater than that of the control irradiated mice. These results suggested that in the IL-1 treated mice splenic function is still dedicated to myelopoiesis and that this is at the expense of lymphopoiesis. Indeed, cytological examination of the spleen cells revealed a significantly greater number of cells of the myelocytic lineage and a paucity of lymphocytes in the IL-1 treated mice compared to irradiated controls (data not shown) [7]. To control for the possible effects of altered homing patterns in the IL-1 treated mice, the number of lymphocytes were assessed in the peritoneal cavity, blood and lymph nodes of the irradiated mice. The results of this, shown in Table 5, revealed that the IL-1 treated irradiated mice had an overall deficit in lymphocyte numbers. Additional studies using immunofluorescent staining and flow cytometry showed that both B cell and T cell numbers were depressed (data not shown).

Table 4. Proliferative response of spleen cells 20 days after exposure to 750 R*.

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

* 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 cpm for the IL-1 treated mice, 9,063 for the MSA-treated mice and 4,351 for the unirradiated control.

Table 5. Quantitation of the lymphocyte population 20 days after 750 R*.

Irradiated mice treated with	No. of lymphocytes (cells $\times 10^{-6}$) in:			
	Spleen	Peritoneal cavity	Blood	Node
MSA	49.8	0.22	0.145	6.0
IL-1	6.4	0.11	0.074	3.4

* 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. The axillary, inguinal, and mesenteric lymph nodes were dissected and pooled for the estimation of their cellularity.

This observation lead to the investigation of the level of lymphopoiesis in the IL-1 treated mice. B cells are formed in the bone marrow, progressing through at least one easily identifiable stage of differentiation characterized by the expression of the B220 cell surface antigen on surface IgM negative cells [16]. Thus, the level of B cell lymphopoiesis could be assessed by determining the number of these cells in the bone marrow. This was done by immunofluorescent staining and flow cytometric analysis. As can be seen in Table 6, bone marrow cells from the irradiated, IL-1 treated mice had significantly fewer numbers of B220$^+$ cells than did the irradiated control mice. In contrast, the level of Mac 1$^+$ cells (a marker on macrophage/granulocyte precursor cells as well as mature macrophages) [17] was significantly increased in the IL-1 treated mice. Thus, it appeared that the bone marrow of the IL-1 treated mice was strongly committed to myelopoiesis and that this was at the expense of B lymphopoiesis.

The vast majority of T cells require a period of intrathymic residence before reaching functional maturity. Thus, the cellularity of the thymus was studied in the mice following irradiation as an indice of T cell growth and development. As can be seen in Figure 6, the cellularity of the thymus was severely depleted as a consequence of the irradiation. Thymic cellularity increased with time in the control irradiated mice, but surprisingly never rebounded in the IL-1 treated mice. In

Table 6. Proportions of B220$^+$ and Mac-1$^+$ cells in the bone marrow 20 days after irradiation.

Source of bone marrow cells	Cellularity ($\times 10^{-6}$)	Percent cells staining positively for:	
		Mac-1	B220
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)

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 B220 markers ($p < 0.01$). The data obtained from the normal mouse is representative of the values from unmanipulated mice.

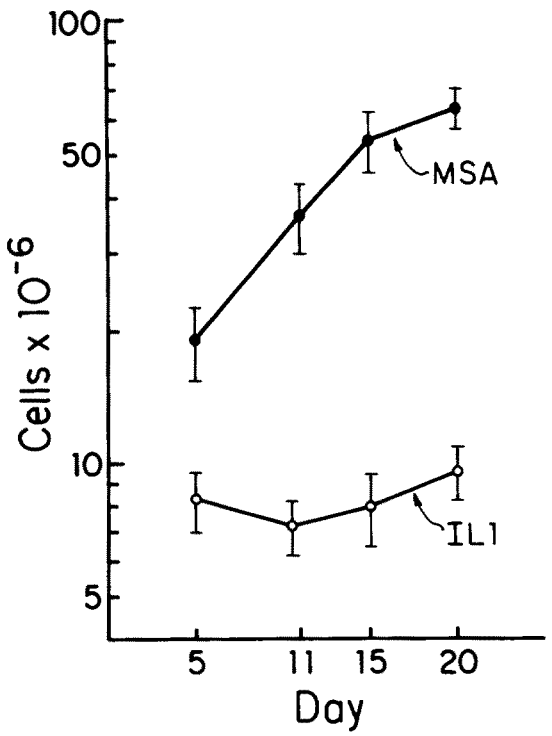

Fig. 6. Effect of IL-1 therapy after 750 R on the regeneration of thymic cellularity. Irradiated mice were injected twice daily with IL-1 or MSA (200 ng/injection). At the indicated time, the mice were killed, the thymus dissected and teased into a cell suspension, and the cells counted. The data are the mean ($\pm$ SEM) of four individually assayed mice. The difference in cellularity is statistically significant on days 11, 15 and 20 ($p<0.01$).

these mice, the thymus remained chronically hypoplastic for the duration of the experiment. Thus, it was possible that the deficit of T cell numbers seen in the IL-1 treated, irradiated mice was a consequence of diminished thymic processing.

Discussion

The regulation of hematopoiesis is clearly a complex event which can be mediated by a number of well characterized CSF. It is now clear that other cytokines or interleukins such as IL-3, IL-4, and IL-6 can also influence hemopoiesis [18–20]. One such entity, originally described by Stanley, was termed hemopoietin 1 [2]. H-1 acted to upregulate the expression of CSF-1 receptors in bone marrow depleted of cycling cells by 5-FUra treatment.

In our studies on the characterization of H-1, it was found that it was biochemically and serologically indistinguishable from IL-1α [6]. Thus, attempts to resolve IL-1 bioactivity from the potentiation of CSF activity using three different chromatographic procedures were not successful.

It was also observed that the HBT 5637 cell line expressed the genes for IL-1α and IL-1β and that the conditioned medium had IL-1 activity as assessed in the IL-1 bioassay. Also, when tested in the CSF proliferation assay, it possessed strong activity. Finally, the critical evidence in determining that H-1 was indeed IL-1 was the ability of an anti-IL-1α monoclonal antibody to precipitate the CSF potentiating activity of the HBT 5637 conditioned medium. Thus, the overall weight of evidence strongly suggested that the CSF potentiating activity in the conditioned medium of the HBT 5637 cell line was indeed attributable to IL-1.

Therefore, IL-1 can play an important role in the process of hematopoiesis. It appears that the action of IL-1 is at least bifunctional. First, IL-1 might have a direct effect on earlier stem cells causing them to express receptors for CSF and become CSF responsive [2] and second, to induce the production of CSF from bone marrow stromal cells [21, 22]. As of yet it is not clear if IL-1 is a requisite factor for the maintenance of normal hematopoiesis or represents a mechanism by which hematopoiesis may be accelerated in crisis situations. However, a recent study using the technique of *in situ* hybridization has produced evidence that the IL-1 gene is constitutively expressed in the bone marrow of normal mice suggesting that IL-1 may be involved in the homeostatic regulation of hematopoiesis [23]. In addition, in binding studies using radiolabeled IL-1α, we had determined that both a primary murine myeloid

bone marrow population which was 98% Mac-1 positive and a murine factor dependent cell line FDCP-2, expressed receptors for IL-1α (DYM, unpublished observations).

The potential use of IL-1 as a therapeutic agent to enhance hematopoiesis was tested in mice exposed to a sublethal dose of irradiation (750 R) [7]. In this model system, 100% of the mice survive but the full recovery of the hematopoietic system and peripheral neutrophil numbers extends over thirty days. In the experiments presented here IL-1 was administered to the mice twice daily in an effort to accelerate hematopoietic reconstitution. Following irradiation, IL-1 treatment caused a more rapid recovery of bone marrow colony forming capacity. This was most significant at the early time points following radiation indicating that IL-1 administration could cause a rapid expansion of colony forming cells after the cytoreductive effects of whole body radiation. In addition, peripheral blood neutrophils also showed an accelerated recovery rate with significantly higher levels on day 15 and 21 post irradiation. That this increase in peripheral blood neutrophils reflected an increased in host resistance to infection was borne out by greater median survival time of the IL-1 treated mice following a lethal challenge with *S. typhimurium*. Thus, treatment of mice with IL-1 following sublethal whole body irradiation significantly accelerated myelopoietic recovery resulting in an increased resistance to infectious disease.

In contrast to the beneficial effects of IL-1 treatment on myeloid reconstitution were the findings on the recovery of the immune system. Lymphocytes are generally considered to be very radiosensitive [14], and a dose of 750 R eliminates well over 95% of both B cells and T cells. Treatment of the mice with twice daily injections of IL-1 resulted in a general lymphopenia. Spleen cell responses to T cell and B cell mitogens (Con A and LPS) were significantly less than that of the irradiated control mice and the thymus was chronically hypoplastic. The number of $B220^+$ cells (a marker expressed by pre-B cells) in the bone marrow was also significantly depressed. Thus, both B cell and T cell development were negatively affected by IL-1 administration. The reasons for this are not yet entirely clear, but it appears that the levels of hydrocortisone induced by IL-1 are sufficient to cause thymic hypoplasia [24]. It has been observed that following irradiation, bone marrow derived pre-thymocytes are able to seed the thymus in IL-1 treated mice, but that the $CD4^+/CD8^+$ thymocyte subpopulation fails to develop [24]. This is the population which is predominantly affected by hydrocortisone [25]. Thus, it appears that the thymic hypoplasia is not a direct consequence of IL-1 action but secondary to increased levels of hydrocortisone. The decreased numbers of $B220^+$ cells in the bone marrow of the IL-1 treated mice could be due to simple crowding effects by the increased commitment to myelopoiesis or by direct inhibitory effect of IL-1. This observation has been corroborated *in vitro* by Dorschkind who studied long term bone marrow cultures and found that the addition of IL-1 prevented B lymphopoiesis and maintained myelopoiesis [26]. This process could be mimicked by the addition of exogenous CSF to the cultures suggesting that the induction of CSF production by IL-1 contribute to an environment which is suboptimal for B lymphopoiesis. thus, prolonged IL-1 treatment following cytoreductive modalities might prolong the time required to achieve immune competence.

The therapeutic potential of IL-1 is broad due to its central involvement in the acute phase response, T cell and B cell activation and, as demonstrated here, hematopoiesis. For instance, IL-1 has been recently shown to enhance resistance to infectious disease in mice made neutropenic by cyclophosphamide [27–29] as well as augmenting resistance in normal animals [30, 31]. Thus, *in toto*, these data convincingly indicate that for hastening the recovery of the myelopoietic system from radiation of chemotherapy, IL-1 shows promising potential.

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