Prevention of Doxorubicin-induced Hematotoxicity in Mice by Interleukin 1

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ABSTRACT
Interleukin 1α and interleukin 1β induce peripheral neutrophilia with stimulation of granulopoiesis in bone marrow. The continuous administration of interleukin 1 (100 ng/day) to mice for 7 days by s.c.-implanted Alzet osmotic minipumps induced marked stimulation of granulopoiesis in marrow and spleen in normal mice, and protected against the marked depletion of myeloid and erythroid cells in bone marrow of mice treated with single injections of either 20 or 30 mg/kg doxorubicin (DXN). Interleukin 1β infusion also protected against DXN-induced atrophy of thymus and secondary lymphoid organs. Single i.p. injection of either interleukin 1α or interleukin 1β at doses up to 1000 ng 24 h prior to treatment with DXN did not protect against the hematopoietic and lymphoid toxicities of DXN.

MATERIALS AND METHODS
Mice. Female C57BL/6J mice (16–18 g) were purchased from The Jackson Laboratory, Bar Harbor, ME.
Reagents and Treatments. Doxorubicin hydrochloride (DXN; Adriamycin) was purchased from Sigma Chemical Co., St. Louis, MO, or from Adria Labs, Inc., Columbus, OH. DXN was dissolved in 0.85% NaCl solution and administered to mice as a single (20 or 30 mg/kg) i.p. injection. Recombinant human IL-1α and IL-1β were obtained from Immunex, Seattle, WA, and were determined to contain 40 pg endotoxin/μg protein and 3 pg endotoxin/μg protein, respectively, by the Limulus amebocyte lysate test. The IL-1α and IL-1β preparations each contained approximately 2 × 10³ thymocyte units IL-1 activity/mg protein, where one unit results in 50% of maximum proliferation of thymocytes obtained from 5- to 8-week-old C3H-HEJ mice (10). Interleukin-1α and IL-1β were prepared in 0.85% NaCl solution (with 0.1 mg/ml mouse serum albumin added for stability). Doses of 1, 10, 100, or 1000 ng/day for 7 days were infused s.c. into the mice via osmotic minipumps (Model 2001; Alza Corp., Palo Alto, CA), or as a single i.p. dose of 100 or 1000 ng/day. All treatments with IL-1α or IL-1β were initiated 24 h prior to DXN treatments.
Pathological Evaluations. Blood samples were collected by cardiac puncture after anesthesia and necropsy examinations were performed immediately after death. Tissues were preserved in neutral buffered 10% formalin and processed for light microscopy by standard procedures. Sections were stained with hematoxylin & eosin. Tissues examined included sternum for section of bone marrow, spleen, thymus, mesenteric lymph node, liver, heart, kidneys, and lung. Bone marrow smears were prepared at necropsy, fixed in methanol and stained with Wright Giemsa stain.
Sections of bone marrow and spleen were graded histologically for the degree of hematopoietic activity. Bone marrow sections from treated animals were compared to sections from control animals and the relative increase or decrease in granulopoiesis, erythropoiesis or numbers of megakaryocytes was scored as follows: −, no change from control; ±, minimal increase or decrease in some but not all animals in the group; + to +++++, relative values assigned by visual inspection for minimal to severe increase or decrease compared to controls. For increased (+) cellularity, + = 10–20% increase over control; ++ = 2× increase; +++ = 3× increase; +++++ = 4× increase. For decreased (−) cellularity, + = 80% of control; ++ = 50% of control; +++ = 20% of control; +++++ = 10% of control; ++++++ = less than 5% of control.
Spleen sections of control mice had little evidence of EMH. Spleen sections from treated mice were scored for increases in extramedullary granulopoiesis or erythropoiesis and numbers of megakaryocytes as compared to controls. The relative values for the grades used for spleen sections were: + = 2× increase; ++ = 5× increase; +++ = 10× increase; +++++ = 20× increase; ++++++ = 30× increase compared to controls.

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1 To whom requests for reprints should be addressed, at Syntex Corporation, 3401 Hillview Avenue, Palo Alto, CA 94303.
2 The abbreviations used are: IL-1, interleukin 1; GM-CSF, granulocyte-macrophage colony stimulating factor; DXN, doxorubicin; IL-2, interleukin 2; EMH, extramedullary hematopoiesis.
In addition, lymphoid atrophy or hyperplasia in sections of thymus, spleen, and lymph nodes was scored for severity with (+ to +++++), minimal to severe, respectively, based on histopathological criteria for these changes.

Hematology measurements were performed on an ELT-8 Hematology Analyzer (Ortho Instruments, Westwood, MA). Differential leukocyte counts were determined by visual appraisal of Wright Giemsa-stained blood smears.

RESULTS

Dose Response. The effects of graded doses of IL-1β delivered by continuous 7-day s.c. infusion on peripheral blood differential counts are illustrated in Fig. 1. Interleukin-1β induced a relative peripheral neutrophilia and lymphopenia at a dose level as low as 100 ng/day, with a dose-dependent increase at 1000 ng/day. A slight increase in percentages of band neutrophils was observed at all doses tested.

Histopathological changes in hematopoietic and lymphoid tissues are shown in Table 1. The response to IL-1β in the bone marrow was primarily stimulation of granulopoiesis at doses that produced changes in the peripheral blood, as evidenced by increased numbers of myeloid precursor cells. Increased EMH, predominantly granulopoiesis, was observed in spleen with a dose-related increase in severity. Megakaryocytes were also increased in bone marrow and spleen. Slight stimulation of erythropoiesis was observed in the spleen; however, a minimal decrease in erythroid precursor cells was observed in the bone marrow of mice receiving IL-1β at 1000 ng/day.

The IL-1β infusion at 1000 ng/kg produced mild thymic atrophy characterized by depletion of cortical thymic lymphocytes. Lymphoid hyperplasia was also observed in lymph nodes at that dose.

Necrosis and inflammation were observed in the skin overlying the drug delivery portal of the osmotic pumps in mice receiving 1000 ng/day. Therefore, a dose of 100 ng/day was selected as the optimal IL-1β dose which produced stimulation of hematopoietic tissues without producing unacceptable toxicity, and that dose was used in subsequent studies.

Comparison of Single Injection and Infusion of IL-1α and IL-1β. The hematological (Table 2) and morphological (Table 3) changes were similar in mice treated with either IL-1α or IL-1β by continuous infusion for 7 days. In both groups there was an absolute neutrophilia with lymphopenia, with no change in total leukocyte values. Erythrocyte counts, hematocrits, and hemoglobin values were slightly decreased, and platelet counts were increased. These changes in the peripheral blood were accompanied by an increase in granulopoiesis in bone marrow (Fig. 2), spleen, and liver, and a slight suppression of erythropoiesis in bone marrow. A slight stimulation of erythropoiesis was evident in the spleen. Megakaryocytes were increased in bone marrow and spleen. Minimal thymic atrophy was observed with both IL-1α and IL-1β infusion.

Mice that received a single i.p. injection of 100 ng of either IL-1α or IL-1β had no changes in hematological parameters, and only minimal stimulation of bone marrow granulopoiesis when examined 7 days after injection. At 1000 ng by single i.p. injection, minimal stimulation of both granulopoiesis and erythropoiesis was observed in bone marrow and spleen with both IL-1α and IL-1β. No effect was observed in the thymus with a single dose of either interleukin.

Moderate lymphoid hyperplasia was observed in lymph nodes and spleen of mice treated with both IL-1α and IL-1β, whether by infusion or a single i.p. injection.

Effect of Infusion of IL-1α or IL-1β on Hematopoietic Toxicity of Doxorubicin. DXN was administered at two doses: 20 mg/kg, i.p., which approximates the LD₅₀ value for these mice, or 30 mg/kg, i.p. (Table 4). At 20 mg/kg, DXN produced mild stimulation of granulopoiesis in bone marrow but not spleen by Day 7. The degree of granulopoiesis in bone marrow was markedly enhanced in mice receiving IL-1α or IL-1β, 100 ng/day for 7 days by s.c. infusion beginning 24 h prior to administration of DXN, and was increased over that observed with either DXN or IL-1 treatment alone. The bone marrow changes were accompanied by a neutrophilia and lymphopenia in the peripheral blood which was comparable to that observed in mice receiving infusion of IL-1 only (data not presented). Mice receiving IL-1α in addition to DXN (20 mg/kg) had a marked decrease in erythropoiesis in bone marrow, which was not observed with DXN alone or IL-1β plus DXN. A mild increase in EMH was observed in the spleens of mice receiving the infusion of IL-1β in addition to DXN, but it was not comparable

### Table 1 Effect of dose of continuous IL-1β infusion on hematopoietic and lymphoid tissues in vivo

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<td>&lt;E</td>
<td>&lt;E</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

* C57Bl/6J mice (n = 5/group) received continuous infusion of IL-1β from s.c.-implanted osmotic minipumps at a rate of 8 μL/h for 7 days. The control group animals received 0.9% NaCl solution.

a Histopathological severity scores: -, not present; ±, minimal in some, but not all, of the mice in the group; Bone marrow increased (>) cellularity: + = 10-20% increase; ++ = 20-40% increase; +++ = 40-60% increase; ++++ = 60-80% increase; ++++ = >80% increase. Bone marrow decreased (<) cellularity: +/- = <5% of control; ++ = 5-10% of control; +++ = 10-20% of control; ++++ = 20-30% of control; ++++ = >30% of control.

b Symbols: >, increased; <, decreased.
to that observed in mice treated with IL-β only (Table 3).

At 30 mg/kg, DXN produced marked depletion of both myeloid and erythroid cells in bone marrow (Fig. 2). Concurrent infusion of IL-1β protected against the bone marrow effects, and resulted in increased granulopoiesis in the marrow comparable to that obtained with IL-1α alone (Fig. 2). Moderate stimulation of EMH was also observed in spleens of mice treated with the IL-1β in addition to DXN (30 mg/kg).

Thymic atrophy characterized by depletion of cortical thymic lymphocytes was produced by DXN at doses of 20 or 30 mg/kg. IL-1β partially protected mice from the thymic lymphoid depletion, but no protection was observed with IL-1α infusion. IL-1β also protected mice from atrophy of secondary lymphoid tissues of spleen and lymph node which was produced by DXN at 30 mg/kg.

There were no lesions present in other tissues, including liver, heart, kidney and lung, that were attributed to acute effects of DXN.

Effect of Single Injection of IL-1α or IL-1β on Hematotoxicity of Doxorubicin. The mice treated with a single injection of IL-1α or IL-1β 24 h prior to DXN treatment had no evidence of protection from the hematotoxicity (Table 5). Mild to moderate stimulation of granulopoiesis in bone marrow was observed in mice receiving IL-1α prior to DXN at 20 mg/kg, which was comparable to that observed in mice receiving DXN alone. At 30 mg/kg DXN, with or without prior IL-1 treatment, there was a marked decrease in both erythroid and myeloid precursor cells in marrow sections.

Thymic atrophy was severe in all groups, although partial protection from the atrophic changes in secondary lymphoid tissues of spleen and lymph node was observed with both IL-1α and IL-1β administered in single doses of 1000 or 100 ng, respectively.

DISCUSSION

Our results demonstrate that both IL-1α and IL-1β when infused via 7-day Alza minipumps produced comparable stimulation of hematopoiesis and lymphoid hyperplasia in mice. Such an IL-1 infusion initiated 1 day prior to doxorubicin injection protected mice from the severe myelotoxicity of DXN and resulted in overall increased hematopoiesis in bone marrow and spleen such as obtained in IL-1 only treated mice. It is noteworthy that a single injection of IL-1α or IL-1β, given 24 h prior to the DXN, did not protect from the DXN-induced hematotoxicity, even when doses up to 1000 ng of IL-1 were given. This finding is quite different from the effects of IL-1 on radioprotection, in which a single injection of 100 or 200 ng
INTERLEUKIN 1 AS A HEMATOPROTECTOR

Fig. 2. Photomicrographs of bone marrow of mice. H & E, x 300. a control animal infused with saline for 7 days. Marrow demonstrates normal cellularity and approximate equal proportions of myeloid and erythroid precursor cells. b, animal infused with IL-1β (100 ng/day) via s.c. osmotic minipump for 7 days. This marrow has a moderate increase in cellular density with increased myeloid cells and a slight reduction in erythroid cells. c, animal received DXN (30 mg/kg) i.p. 6 days prior to necropsy. Hypocellular marrow with distended, blood filled sinusoids. d, animal received IL-1β (100 ng/day) via s.c. infusion for 7 days and DXN (30 mg/kg) i.p. on Day 2. This marrow has a marked increase in cellular density with a predominance of myeloid cells and very few erythroid cells present.

Table 4 Comparison of effect of continuous IL-1α or IL-1β infusion on hematotoxicity of doxorubicin in mice

<table>
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<th>Tissue/lesion</th>
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<th>30 mg/kg</th>
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<td></td>
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<td></td>
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<tr>
<td>Lymph nodes</td>
<td>Atrophy</td>
<td>NE&lt;sup&gt;d&lt;/sup&gt;</td>
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</table>

* Single i.p. injection of DXN was given 24 h after implanting the osmotic minipumps. IL-1α or IL-1β was administered by continuous infusion at a dose of 100 ng/day for 7 days (n = 3/group).
<sup>e</sup> Symbols: (>, increased; (<, decreased.
<sup>f</sup> Histopathological severity scores same as in Table 1.
<sup>d</sup> NE, not examined.

IL-1α or IL-1β, respectively, was sufficient to protect approximately 80% of the mice from radiation-induced death (4). The occasional differences in responses to IL-1α and IL-1β which we observed (Tables 3 and 4) may reflect pharmacokinetic differences of these two forms of IL-1, but more extensive studies would be needed to address this issue.

In order to initially define how the IL-1 resulted in this hematopoietic protection, we studied the cellular changes in-
duced in the various hematopoietic and lymphoid tissues. The results obtained with IL-1 alone correlated with the protective effects observed. Thus 7-day s.c. infusion of IL-1 via miniosmotic pumps resulted in stimulation of hematopoiesis by Day 7. This was characterized by markedly increased granulopoiesis and slightly depressed erythropoiesis in bone marrow, accompanied by increased extramedullary hematopoiesis in spleen and liver. Although total WBC counts remained unchanged, the ratio of neutrophilia and lymphopenia following i.v. injection (11), which is accompanied by the selective release of mature neutrophils from the marrow (12). Interleukin-1 also enhances granulopoiesis by inducing cytokines required for myeloid proliferation (13, 14). The mechanism for the depression of erythropoiesis with the IL-1 treatment is not known; however IL-1 has been shown to inhibit the action of erythropoietin on erythroid precursors in the marrow (15).

We observed a slight atrophy of the thymus after 7-day infusion of either IL-1α or IL-1β (Table 3); thymic atrophy was also observed by Morrissey et al. (6) after multiple daily injections of mice with IL-1α. However, it is noteworthy that the severe thymic atrophy induced by DXN treatment was partially reversed by the IL-1β infusion (Table 4). In contrast, Morrissey observed an increase in thymic cellularity with time after irradiation in control mice and no such increase in irradiated mice receiving IL-1α treatment (6).

Mice receiving the 7-day infusion of IL-1 were protected from the acute hematotoxicities of DXN alone. However, mice receiving a single injection of IL-1α or IL-1β showed only minimal effects on hematopoietic and lymphoid tissues, and were not protected from the hematotoxicity of DXN.

IL-1 has been shown to regulate the development of primitive hematopoietic cells in concert with lineage-specific colony stimulating factors (GM-CSF or CSF-1) (16). This activity was previously attributed to hemopoietin-1 (17), which now appears to be the same as IL-1 (16). In light of the above, it is significant that IL-1 also stimulates production of GM-CSF from vascular endothelial cells (18, 19) and from fibroblasts (20). GM-CSF stimulates granulocyte and monocyte colony formation (21) and thus probably plays a significant role in resistance to infections (22). Continuous infusion of GM-CSF stimulated hematopoiesis in monkeys (23) and hamsters (24), and shortened the neutropenic period in monkeys given autologous bone marrow transplantation (25). Fourteen-day infusion of GM-CSF into AIDS patients resulted in an increase in circulating neutrophils, eosinophils, and monocytes (26). Thus GM-CSF as a single agent may be useful in hematopoietic restoration. Furthermore, since IL-1 can synergize with GM-CSF in stimulating primitive hematopoietic cells (16), combination of these two agents may be especially beneficial in hematopoietic regeneration. Moore and Warren (8) have recently shown synergy of IL-1 and G-CSF in stimulating stem-cell recovery and hematopoietic regeneration in mice following treatment with 5-fluorouracil. Furthermore, the ability of IL-1 treatment to increase platelets in peripheral blood (Table 2) and megakaryocytes in bone marrow and spleen (Tables 1, 3, and 4) may be additionally beneficial for use in conjunction with cancer chemotherapies which induce thrombocytopenia in addition to neutropenia. However, as IL-1 is also a mediator of inflammation (27), a cautionary note should be observed in exploring its therapeutic potential.

REFERENCES


Table 5: Effect of single injection of IL-1α or IL-1β on hematotoxicity of doxorubicin in mice

<table>
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* NE, not examined.
* Symbols: (>), increased; (<), decreased.
* Histopathologic severity scores same as in Table 1.


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