Reduction of Radioantibody-induced Myelotoxicity in Hamsters by Recombinant Interleukin-1

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ABSTRACT

Recombinant human interleukin 1 (IL-1) administered i.p. to tumor-bearing hamsters results in a dose-dependent 2- to 5-fold increase in total peripheral white blood cells. These levels remain elevated for 2 to 3 wk and then decline to base-line levels. Pretreatment of animals with similar doses of IL-1 prevents radioimmunotherapy-induced destruction of the radiosensitive hematopoietic system. Furthermore, recovery from radioimmunotherapy-induced myelosuppression is possible if animals are given IL-1 1 wk after radioimmunotherapy treatment. Thus, both protection and rescue from the hematopoietic damage associated with radioantibody treatment are feasible by pre- or posttreatment with IL-1.

INTRODUCTION

Exposure to radiation results in damage to the hematopoietic system. Mitotically active precursor cells are depleted by low levels of radiation, and the subsequent supply of mature cells to the peripheral circulation is diminished. Within a few weeks after irradiation, resistance to specific pathogens decreases (1). Protection and/or recovery from the injurious effects of radiation is therefore of interest (2). Immunomodulators have been reported to confer radioprotection. Numerous microbial components such as lipopolysaccharide, which are now known to enhance hematopoietic and immune functions, were shown to have radioprotective activity more than 30 yr ago (3–5). More recently, addition of cultured monocyte supernatants that contained IL-1, a differentiation- and maturation-inducing agent and one of several lipopolysaccharide-stimulated products, conferred a high degree of radioprotection to human peripheral T-lymphoid populations in vitro (6). Neta et al. (7) have demonstrated that pretreatment of mice with IL-1 increases the percentage of survival of mice in a dose-dependent manner (7). In subsequent reports, they demonstrated that pretreatment of lethally irradiated animals with IL-1 resulted in enlargement and increased cycling of bone marrow cells, and an enhanced recovery of total nucleated bone marrow and hematopoietic progenitor cells. In addition, suspension cultures of these bone marrow cells were more responsive to granulocyte-macrophage colony-stimulating factor (8). The effects of IL-1 are mediated through the induction of colony-stimulating factor (9), one of many hematopoietic growth factors induced by IL-1 stimulation of endothelial cells (10–12).

The use of radionuclide-labeled polyclonal and monoclonal antibodies to tumor-associated antigens has shown promising results for detection of primary and metastatic cancers and is now being evaluated in clinical trials for therapeutic efficacy (13, 14). In animal studies a single dose of 1.0 mCi of 131I-labeled antibody administered to hamsters results in moderate toxicity as measured by a 50 to 60% decline in circulating p-WBCs. In this report, we show that the radioantibody-induced loss of p-WBCs can be prevented by a single injection of endotoxin-free recombinant human IL-1 given 20 h prior to radioantibody injection, or reversed by IL-1 injection 7 days after the 131I-labeled antibody.

MATERIALS AND METHODS

Animal Model. Female Syrian hamsters (10 to 14 wk old) bearing GW-39 tumors, a serially propagated CEA-producing human colonic xenograft (15, 16) in both cheek pouches, were used for these studies. In all studies, animals were transplanted with 0.5 ml of a 20% suspension of GW-39 cells prepared in PBS (0.01 M phosphate:0.15 M NaCl:0.02% NaN3, pH 7.2) 4 to 5 days prior to the injection of the radiolabeled antibody.

Antibody Purification and Radiolabeling. Polyclonal goat anti-CEA was prepared by affinity chromatography using a CEA immunoadsorbent, as described by Primus et al. (17). Antibody purity was tested by immunoelectrophoresis, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of unreduced and reduced samples, and by size exclusion high-pressure liquid chromatography on Micropak TSK-3000 SW (7.5 x 300 mm; Varian, Walnut Creek, CA).

The antibody was radioiodinated with 131I (New England Nuclear, N. Billerica, MA) by the chloramine-T method (18) to a specific activity of 12 to 15 mCi/mg. Unbound radioiodine was separated from antibody-bound iodine by gel filtration over a PD-10 column (Pharmacia) that was equilibrated in 0.04 M PBS, pH 7.4, containing 1% human serum albumin. All radioantibodies were characterized by molecular sieve chromatography over an S-200 column (1.6 x 60 cm; Pharmacia) for immunoreactivity by passage through a CEA immunoadsorbent. In all preparations, there were less than 5% aggregated antibody and less than 3% free iodine. Immunoreactivity of the labeled antibody was in the range of 60 to 75%.

Interleukin Stock. Recombinant human IL-1a (Lot 1-87) was a generous gift from Dr. P. Lomedico of Hoffmann-LaRoche, Nutley, NJ. This preparation has a specific activity of 2.5 x 106 units/mg. This protein was purified from Escherichia coli and consisted of the carboxyterminal 154 amino acids of the 271 amino acids found in the human IL-1α precursor (19). It is essentially a pure protein preparation with only minute amounts of endotoxin present (0.125 EU/ml).

WBC Measurements. Blood from individual anesthesized hamsters was collected into heparinized syringes by intracardial puncture. Aliquots (100 µl) were treated with lysing buffer (8.26 g/liter of ammonium chloride:0.1 g/liter of potassium bicarbonate:0.0379 g/liter of EDTA) for 5 min to remove RBC. Following a 10-min centrifugation at 2800 x g, the supernatants were removed by aspiration, and the cell pellets were resuspended in 2.0 ml of 0.1 M PBS, pH 7.2. The cells were pelleted a second time, the buffer was removed, and the cells were resuspended in 1.0 ml of PBS. The number of WBCs was evaluated by immunofluorescent staining on a FACStar plus (Becton Dickinson, Mountain View, CA). The cell samples were incubated overnight with the anti-CEA antibody (Lot 18465) followed by a fluoresceinconjugated goat anti-rabbit IgG (Becton Dickinson), and a second fluoresceinconjugated goat anti-CEA antibody (Lot 18465). The samples were then analyzed for their fluorescent properties. A total of 1 x 106 cells were counted for each group. Results for each group are expressed as the mean ± SD of triplicate measurements.

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\[ X_{\text{group}} = \sum (n_i/X_{\text{untreated}} + n_j/X_{\text{untreated}} + \ldots \times 100\%) / N \]
where $X$ is the mean for either the untreated or the treated group, $n$ is the p-WBC count for an individual animal within the group, and $N$ is the total number of animals in the group.

Wright’s Staining. Slides with air-dried blood films are covered with Wright’s stain (0.1 g of powder in 60 ml of methanol) for 4 min. An equal volume of Wright’s buffer (6.63 g of KH2PO4: 2.56 g of Na2HPO4, pH 6.4) is added to the slide and allowed to stand for 10 min. The stain is washed off slowly with a stream of distilled water. The slide is air dried, and a cover slip is mounted.

RESULTS

Hamsters bearing a CEA-producing xenograft had an average of 2961 WBC/mm³ (range, 2349 to 3573) at the start of the first study. Over a period of 4 wk, the group mean fluctuated between a low of 2556 and a high of 3280 WBC/mm³ (range, 2274 to 3851). Treatment of these animals with 1.0 mCi of 131I-labeled goat anti-CEA, a dose which is therapeutically efficacious (14), results in a 50% loss in peripheral white blood cells ($X = 1567 ± 571$) within 7 days of injection (Fig. 1). WBC values remain depressed for a minimum of 4 wk before they gradually return to base-line levels 6 to 7 wk later (data not shown). The average WBC count for the 131I-Ab-treated group fluctuated between 763 and 2350/mm³.

The effect on p-WBCs by pretreatment of tumor-bearing animals with 2 to 80 ng of IL-1 alone is shown in Fig. 2. Untreated animals had an average of 2,586 ± 271 WBC/mm³ at the start of this study. Their WBCs fluctuated upward over a 4-wk period to a high of 5,273 ± 849/mm³. IL-1 given at 2 ng/animal did not significantly alter p-WBCs (2544 ± 276/mm³). Higher doses of 20 to 40 ng of IL-1 are able to protect against this loss completely and further elevate p-WBCs above control values (e.g., 199.8% of 8,100 ± 2,377/mm³ with 100% of 10 ng of IL-1 compared to 26.6% of control (in the absence of IL-1 on Day 7 postradioantibody). Higher doses of 20 and 40 ng of IL-1 are able to protect against this loss completely and further elevate p-WBCs above control values (e.g., 199.8% of 8,100 ± 2,377/mm³ with 20 ng and 253.1% or 10,260 ± 827/mm³ with 40 ng on Day 7 postradioantibody).

The injection of 2.0 mCi of radioantibody into these IL-1-pretreated animals resulted in a population of mature p-WBCs. No apparent increase in the number of myeloblasts or myelocytes was noted. Histological evaluation of blood smears stained with Wright’s solution indicated that treatment with 40 ng of IL-1 resulted in a population of mature p-WBCs. No apparent increase in the number of myeloblasts or myelocytes was noted.

Since IL-1 was found to be effective in protecting against myelosuppression with 1.0 mCi of radioantibody, we then investigated whether the elevation in p-WBCs by pretreatment with IL-1 could prevent the myelosuppression resulting from the administration of 1.0 mCi of 131I-labeled antibody (Table 1). Animals that were not pretreated with IL-1 show a typical loss in p-WBCs 7 days after radioantibody (untreated, 4,054 ± 626/mm³; 131I-Ab treated, 1,078 ± 142/mm³). WBCs in the untreated group fluctuated between 4,054 and 6,379/mm³ during the 4-wk experiment. Doses of 10, 20, or 40 ng of IL-1 administered 20 h before radioantibody resulted in a 1.4- to 2.0-fold increase in p-WBCs on Day 0 immediately prior to radioantibody injection (10 ng of IL-1, 5,618 ± 1,930; 20 ng of IL-1, 6,998 ± 2,087; 40 ng of IL-1, 7,993 ± 1,288). In the absence of radioantibody, there was a quantitative dose-dependent increase in p-WBCs over a 4-wk period. A dose of 10 ng of IL-1 partially protects the loss in p-WBCs (e.g., 66.6% of control with 10 ng of IL-1 compared to 26.6% of control (in the absence of IL-1 on Day 7 postradioantibody). Higher doses of 20 and 40 ng of IL-1 are able to protect against this loss completely and further elevate p-WBCs above control values (e.g., 199.8% of 8,100 ± 2,377/mm³ with 10 ng of IL-1 compared to 26.6% of control (in the absence of IL-1 on Day 7 postradioantibody). However, even with 20 to 40 ng of IL-1, the administration of 1 mCi of radiolabeled antibody reduced the number of p-WBCs on Day 7 in comparison to animals given IL-1 but no radioantibody. This reduction was only apparent on Day 7, and by Day 14 the 2 groups of animals had similar numbers of p-WBCs. Whereas 100% of the animals showed a positive response with 20 to 40 ng of IL-1, only 75% of the animals showed a response with 10 ng of IL-1. Cytometric separation of the total p-WBC population into granulocytes and agranulocytes indicated that the granulocytes were initially stimulated to a greater extent than agranulocytes (262 versus 179%) 24 h after IL-1 treatment; however, both populations were equally enhanced 7 days post-IL-1 treatment (data not shown). Histological evaluation of blood smears stained with Wright’s solution indicated that treatment with 40 ng of IL-1 resulted in a population of mature p-WBCs. No apparent increase in the number of myeloblasts or myelocytes was noted.

Our next experiment was designed to address whether ani-
4. A dose of 1.0 mCi of radioantibody resulted in a 43% decline in p-WBCs in animals that were not treated with radioantibody and received IL-1 on Day 7. Subgroups, those receiving 40 ng of IL-1 on Day 7 post-radioantibody and those left untreated. The p-WBCs in these two groups were compared to a group of animals that did not receive IL-1 or radioantibody treatment. IL-1 can also be used to rescue animals that have been exposed to radioantibody and already show a reduced number of circulating WBCs. Consideration of whether other factors may contribute to the reduction in WBCs is supported by data showing that IL-1 stimulation of p-WBCs with IL-1 and radioantibody beyond the radioprotective effect.

**DISCUSSION**

Radioimmunotherapy with 131I-labeled antibody in the hamster model is limited to a 1.0-mCi single dose of radiiodine per animal due to the toxic effects on the quantity of circulating p-WBCs and on body weight. In this paper, we show that recombinant IL-1, a homogenous product devoid of other biologically active cytokines (19), can be used to stimulate the number of total white blood cells in the peripheral circulation.

Furthermore, pretreatment of animals with IL-1 will protect them from myelotoxicity resulting from radioimmunotherapy. IL-1 can also be used to rescue animals that have been exposed to radioantibody and already show a reduced number of circulating WBCs. These results are particularly important, since they provide a method of reducing one of the key forms of toxicity associated with radioantibody therapy and may now permit the use of higher doses of radioantibody. Although myelotoxicity may be partially reduced by IL-1 treatment, we appreciate the increased risk of toxicity to other organs if higher doses of radioantibody are given. Thus, further studies will be necessary to determine other measures of toxicity.

The multiplicity of effects associated with IL-1 is well documented (20). Cell targets include not only T- and B-cells and myeloid cells, but mesenchymal, neuroectodermal, and hepatic cells as well. However, the rapid clearance of IL-1 from the circulation (21) reduces the likelihood that this cytokine would exert harmful long-term effects on the host. Indeed, there may be additional benefits to the combination of IL-1 with radioantibody beyond the radioprotective effect.

The radioprotective effect of IL-1, as measured by the percentage of animals surviving a lethal dose of ionizing radiation, has been documented recently (7). A variety of mechanisms have been suggested to account for this radioprotective effect (8, 9, 22, 23). One possible explanation involves a role for IL-1 in the proliferation and differentiation of hematopoietic cells (8). Numerous independent investigators have shown that IL-1 is able to stimulate the production of colony-stimulating factors (8, 9, 22, 23). One possible explanation involves a role for IL-1 in creating a hypoxic milieu for blood cells (23) or a hypoxic internal environment for neutrophils (24), rendering these cells more radioresistant.

The future for radioimmunotherapy will involve the use of multiple dosing with radioantibody. Therefore, we are currently exploring the effectiveness of IL-1 stimulation of p-WBCs with repeated treatments of radioantibody. The ultimate goal will be to optimize the treatment schedule of IL-1 and radioantibody to produce maximal tumor therapy and minimal hematopoietic toxicity.

In addition to its effects on the hematopoietic system, IL-1 has been shown to stimulate host defense against tumors (28–33). The mechanism for the cytotoxic effect is unclear, but it may involve induction of IL-2 and interferon by T-cells (31). Alternatively, IL-1-induced cytotoxicity may be mediated by prostaglandin E secretion from macrophages. This hypothesis is supported by data showing that IL-1-stimulated tumor tox-

### Table 1 p-WBC in hamsters treated with IL-1 in addition to or without 131I-Ab (% of untreated animals)

<table>
<thead>
<tr>
<th>IL-1 (ng)</th>
<th>Day 0</th>
<th>131I-Ab</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100.0 ± 16.1</td>
<td>+</td>
<td>100.0 ± 17.5*</td>
<td>100.0 ± 36.6</td>
<td>100.0 ± 26.7</td>
<td>100.0 ± 2.8</td>
</tr>
<tr>
<td>10</td>
<td>136.1 ± 46.0</td>
<td>+</td>
<td>161.0 ± 32.2</td>
<td>173.3 ± 82.3</td>
<td>132.6 ± 60.1</td>
<td>70.0 ± 9.7</td>
</tr>
<tr>
<td>20</td>
<td>178.0 ± 49.5</td>
<td>+</td>
<td>414.7 ± 66.5</td>
<td>535.9 ± 370.5</td>
<td>167.2 ± 71.4</td>
<td>133.9 ± 11.9</td>
</tr>
<tr>
<td>40</td>
<td>195.6 ± 31.0</td>
<td>+</td>
<td>380.2 ± 63.6</td>
<td>428.7 ± 189.0</td>
<td>319.6 ± 230.9</td>
<td>167.0 ± 25.7</td>
</tr>
</tbody>
</table>

* Mean ± SD.
icity was inhibited by indomethacin (29). Furthermore, exogenously administered prostaglandins E₁ and E₂ promoted cytotoxicity (29). Whether IL-1’s mechanism is mediated by another messenger or involves a direct effect on tumor cells (32), the therapeutic efficacy of IL-1 given alone or in combination with labeled or unlabeled antibody may be a novel form of tumor therapy. The advantage of additive forms of therapy in the absence of problems associated with myelosuppression is promising.

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REFERENCES


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