Use of Hematopoietic Growth Factors to Control Myelosuppression Caused by Radioimmunotherapy

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Abstract

Therapeutically efficacious doses of 131I-antibody result in a loss in circulating white blood cells; the granulocyte population is suppressed by 80-85% and the agranulocytes by 60-65% following 2 mCi of 131I-antibody in hamsters. The administration of 100,000 units of human recombinant interleukin 1 24 h prior to radiolabeled antibody can prevent the loss in WBC from 1 mCi of radioantibody and reduce the loss from 2 mCi of antibody. Recombinant murine granulocyte-macrophage colony-stimulating factor is also a potent stimulator of myelopoiesis and may also be useful as a method of reducing radioantibody-induced myelosuppression. The tumor uptake of radioantibody in animals treated with recombinant interleukin 1 is reduced by 30% 1 day after injection of radioantibody but returns to levels seen in animals not treated with the cytokine at 96 and 168 h. Therapeutic efficacy is not compromised by doses of interleukin 1 used to prevent myelosuppression. Therefore, the use of cytokines will permit the use of higher doses of radioantibody for greater tumor therapy with less myelotoxicity than in the absence of cytokine treatments.

Introduction

Radioantibody-mediated tumor regression has been reported for numerous tumor xenograft models (1-4). Clinical trials with RAIT have also been initiated by several investigators (5, 6). Radiolabeled antibodies are more advantageous than drug and toxin conjugates because radioantibodies need not be internalized in order to mediate cell killing, nor must they bind to every tumor cell to be therapeutically efficacious. α- and β-emitters can kill at distances of 50 or more cell diameters (7). It is this property, however, that also constitutes the greatest potential limitation to RAIT, namely toxicity to adjacent normal tissues. Mitotically active precursor cells in hematopoietic tissues are depleted by low levels of radiation. The supply of mature cells to the peripheral circulation is then diminished and resistance to pathogens decreases within weeks after irradiation (8). We have observed a 50-85% reduction in pWBC lasting 5-9 weeks following a single dose of 1-3 mCi of 131I-labeled intact antibody in the Syrian hamster. Dosimetric evaluation in one reported animal study indicated that a therapeutically effective dose of 131I-antibody yielded a radiation dose of 16.3 and 32.3 Gy to marrow and spleen, respectively. Mild to severe leukopenia has also been reported in clinical trials by Ettinger et al. (10) and Larson et al. (11).

One approach to reduce host myelotoxicity involves the use of immunomodulators which have been reported to confer radioprotection. Thirty years ago, lipopolysaccharide, a microbial component, was shown to enhance hematopoietic and immune functions (12, 13). More recently, IL-1, a lipopolysaccharide-stimulated product, conferred radioprotection in part by NIH Grant CA39841.

Materials and Methods

Antibody Purification and Radiiodination. Goat anti-CEA antibody was prepared by affinity chromatography using a CEA immunoabsorbent, as described elsewhere (25). Purity was assessed by isoelectrofocusing, sodium dodecyl sulfate-polyacrylamide gel electrophoresis of reduced and unreduced samples, and size exclusion high pressure liquid chromatography on Micropak TSK-3000 SW (7.5 x 300 mm; Varian, Walnut Creek, CA).

Properties of GS-CSF. Recombinant human IL-1 (lot 1-87) was a gift from Dr. R. D. Blumenthal, R. M. Sharkey, R. Kashi, and D. M. Goldenberg, unpublished results, 1988.

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2 The abbreviations used are: RAIT, radioimmunotherapy; CEA, carcinoembryonic antigen; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-1, interleukin 1; pWBC, peripheral WBC.

Man peripheral T-lymphoid cells (14). In vivo studies demonstrated that IL-1 administration could increase survival time of mice following ionizing radiation (15). In a report by Schwartz et al. (16), pretreatment of mice with IL-1 prior to lethal irradiation resulted in more erythroid colony-forming units, erythroid burst-forming units, granulocyte-macrophage colony-forming cells, and spleen colony-forming units than in saline-treated controls. IL-1-dependent increases in survival following irradiation can be correlated with increases in the total number of colony-forming units as well as increases in bone marrow and peripheral blood cellularity (17). We have recently shown that pre- or posttreatment with IL-1 can be an effective method to prevent or reverse radioantibody-induced myelosuppression, respectively (18). GM-CSF is another cytokine that induces maturation and growth of hematopoietic cells (19-21). GM-CSF has been used effectively to induce recovery from radiation- and chemotherapy-induced hematopoietic suppression (22-24). In this report, we evaluate the effectiveness of using either IL-1 or GM-CSF to ablate RAIT-induced myelosuppression.

Animal Model. Ten- to 14-week-old female Syrian hamsters were implanted with GW-39 tumors, a serially propagated CEA-producing human colon xenograft (27, 28), in both cheek pouches. Transplants were performed with 20% suspensions (w/v) in 0.5 ml phosphate-buffered saline (0.01 M phosphate-0.15 M NaCl-0.02% NaN3, pH 7.2). For biodistribution analysis of radioantibody, the animals were given i.p. injections of 100 μCi of 131I-labeled goat anti-CEA and 25 μCi of 131I-labeled normal goat IgG. At specified time intervals, the animals were sacrificed and radioactivity in the blood, tumor, and organs (liver, spleen, kidney, lung, and muscle) was determined with a gamma scintillation counter. All data were corrected for physical decay and background. The amount of radioactivity in the tumor was quantified using a gamma scintillation counter.

Cytokines. Recombinant human IL-1 (lot 1-87) was a gift from Dr. R. D. Blumenthal, R. M. Sharkey, R. Kashi, and D. M. Goldenberg, unpublished results, 1988.
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P. Lomedico of Hoffmann-LaRoche Inc., Nutley, NJ. The preparation had a specific activity of $3 \times 10^4$ units/mg. The protein was purified from *Escherichia coli*, consisted of the carboxyl-terminal 154 amino acids of the 271 amino acids found in the human IL-1 precursor, and was essentially free from endotoxin (29). A lyophilized preparation of murine GM-CSF (lot 620-029) was made available by Immunex, Seattle, WA. The powder was diluted to the desired concentration with phosphate-buffered saline (pH 7.4) and used the same day.

WBC Measurements and Histological Evaluations. Anesthetized hamsters were bled by cardiac puncture and samples were collected into heparinized syringes. RBC were lysed as described previously (18). WBC were quantitated with an Ortho Spectrum III laser flow cytometer equipped with a microprocessor which records total number of cells and sorts cells by their size and internal complexity into granulocyte and agranulocyte populations. pWBC values of groups of treated animals are reported either as a percentage of the untreated group or as the absolute number of cells/mm$^3$ of blood. Slides were prepared from each blood sample and stained with Wright's dye, and individual leukocyte populations were identified and quantitated.

Results

Recombinant human interleukin administered as a single i.p. injection augments the number of total pWBC in a dose-dependent manner (Fig. 1). This stimulation ranges from 1.6- to 4.0-fold as doses of IL-1 are escalated from 25,000 to 100,000 units of lymphocyte-activating factor activity. A 1.0-mCi dose of $^{131}$I radioantibody results in a 50-55% decline in pWBC within 7 days. A 24-h pretreatment with 25,000 units of IL-1 reduced this pWBC loss to 35%. Higher doses of IL-1 (50,000-100,000 units) elevated WBC 2.0-2.5-fold above the levels observed in untreated animals. Partial protection was achieved (80% of untreated levels) with 100,000 units of IL-1 administered 24 h before a 2.0-mCi dose of $^{131}$I-antibody, which itself resulted in a 75-80% loss in WBC. In addition, administration of IL-1 7 days after radioantibody treatments yielded a partial recovery (25% increase) from myelosuppression (18). Further studies are under way to determine the optimal number of treatments and the optimal schedule of administration of IL-1 for both protection and rescue from radioantibody-induced hematopoietic injury.

Analysis of the subpopulations of WBC affected by radioantibody therapy is shown in Fig. 2. Fourteen days after a 2.0-mCi dose of $^{131}$I-antibody, the circulating granulocytes were reduced by 80-85% and the agranulocytes were reduced by 60-65%. The animals recover from this loss within 6 weeks after the initial 2-mCi dose of radioantibody (4-5 weeks for a 1.0-mCi dose of the same antibody; data not shown). This extended time for recovery from myelosuppression would therefore prevent administration of a second treatment of radioantibody for up to 6 weeks. Furthermore, it becomes essential that the method chosen to increase pWBC levels, either before or after radioantibody therapy, be stimulatory to both granulocytes and agranulocytes.

Fig. 2. Total pWBC, granulocytes, and agranulocytes were monitored by flow cytometry over a 7-week period in groups of 10 animals that were untreated or treated with 2 mCi of $^{131}$I-antibody (Ab). Results are recorded as the absolute number of cells/mm$^3$ in whole blood (mean ± SD [bars]).

Examination of the enhancement of subpopulations of WBC with IL-1 or with GM-CSF is shown in Fig. 3. A 24-h pretreatment with either IL-1 or GM-CSF provides a 2.6-fold or 2.1-fold stimulation of granulocytes, respectively, and both resulted in a 1.8-fold stimulation of agranulocytes after 24 h. Since both cytokines were effective at stimulating both populations of leukocytes, the choice of cytokine for future use will depend on which cytokine is tolerated better on repeated dosing. In addition, we are currently evaluating the stimulatory effective of both cytokines administered simultaneously.

We next assessed the biodistribution of radioantibody as the percentage of injected dose per g tissue and the therapeutic potential of the radioantibody following the administration of IL-1. These results are summarized in Table 1 and Fig. 4. The...
tumor uptake of radioantibody in IL-1-pretreated animals was reduced by 30% 1 day after injection of radioantibody (P < 0.001). However, uptake of antibody by other organs was not affected by IL-1. At later time points of 96 and 168 h, no difference in radioantibody uptake was observed in any of the tissues. The cause for the lower antibody uptake 24 h after radioantibody injection in IL-1-treated animals remains unknown. The total rad dose of tumors was 1381 rads/mCi from untreated animals and 1189 rads/mCi from IL-1-treated animals, a 16% decline. However, this reduction may not have any biological significance, since the therapeutic efficacy of radioantibody is not compromised by IL-1 treatment (Fig. 4).

### Discussion

Myelosuppression is a major side effect associated with patients undergoing either radiotherapy or chemotherapy (30). This effect has also been reported in clinical trials with radioantibody therapy (10, 11). Hematopoietic toxicity can put patients at risk to life-threatening infections, due to reduced WBC. An agent or method capable of preventing hematopoietic injury or accelerating recovery from such injury would clearly be beneficial.

Regulating the proliferation of hematopoietic cells is now possible with the availability of several recombinant colony stimulating factors that have been purified (29, 31, 32). An increased WBC has been observed in both animals and patients following administration of certain glycoproteins (33, 34). A protective or restorative effect by these factors has been observed in both rodent models and patients undergoing irradiation or treatment with cytotoxic chemotherapy (16–18, 35–37).

In this paper, we demonstrate that radioantibody treatment results in a reduction in both the granulocyte and agranulocyte cell populations (Fig. 2) and that recombinant human IL-1 or recombinant murine GM-CSF can be used to stimulate both groups of cells in relatively similar magnitudes (Fig. 3). Furthermore, pretreating animals with IL-1 will protect them from radioantibody-induced myelosuppression in a dose-dependent manner (Fig. 1). Human recombinant IL-1 given 24 h before a 1-mCi dose of radioantibody elevates pWBC to significantly greater levels than in normal, untreated animals, a finding that is consistent with reports using IL-1 with cyclophosphamide therapy (17). We demonstrated previously that IL-1 could also be used to partially “rescue” animals that already had their circulating WBC reduced following radioantibody treatment (18). Neta et al. (38) found that IL-1 was not effective after irradiation treatment at prolonging animal survival because the number of stem cells surviving was probably too small. It is likely that RAIT, with lower but more prolonged doses of radiation, does allow sufficient progenitor cells to be available for IL-1 to be active. At least part of the radioprotective effect of IL-1 (15) is the result of enlargement of bone marrow cells and stimulation of colony-forming cells into the cell cycle which in turn results in increases in the number of progenitor colony-forming cells in bone marrow and spleen that survive irradiation.

### Table 1 Biodistribution of 131I-goat anti-CEA in untreated or IL-1-treated animals

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Injected dose/g</th>
<th>N</th>
<th>24 h</th>
<th>% Injected dose/g</th>
<th>N</th>
<th>96 h</th>
<th>% Injected dose/g</th>
<th>N</th>
<th>168 h</th>
</tr>
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<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>GW-39</td>
<td>1.18 ± 0.47</td>
<td>14</td>
<td>15</td>
<td>2.38 ± 0.31</td>
<td>16</td>
<td>1.18 ± 0.78</td>
<td></td>
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<tr>
<td>Liver</td>
<td>0.58 ± 0.11</td>
<td>7</td>
<td>8</td>
<td>0.24 ± 0.05</td>
<td>8</td>
<td>0.08 ± 0.02</td>
<td></td>
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<td></td>
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<tr>
<td>Spleen</td>
<td>0.54 ± 0.10</td>
<td>7</td>
<td>8</td>
<td>0.26 ± 0.05</td>
<td>8</td>
<td>0.12 ± 0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.74 ± 0.12</td>
<td>7</td>
<td>8</td>
<td>0.28 ± 0.09</td>
<td>8</td>
<td>0.11 ± 0.03</td>
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<tr>
<td>Lungs</td>
<td>0.93 ± 0.42</td>
<td>7</td>
<td>8</td>
<td>0.50 ± 0.06</td>
<td>8</td>
<td>0.17 ± 0.03</td>
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<tr>
<td>Blood</td>
<td>2.29 ± 0.62</td>
<td>7</td>
<td>8</td>
<td>1.21 ± 0.16</td>
<td>8</td>
<td>0.41 ± 0.09</td>
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<td>100,000 units IL-1</td>
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<tr>
<td>GW-39</td>
<td>1.16 ± 0.40</td>
<td>24</td>
<td>36</td>
<td>1.94 ± 0.74</td>
<td>26</td>
<td>1.41 ± 0.94</td>
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<tr>
<td>Liver</td>
<td>0.48 ± 0.11</td>
<td>13</td>
<td>18</td>
<td>0.17 ± 0.05</td>
<td>15</td>
<td>0.08 ± 0.03</td>
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<tr>
<td>Spleen</td>
<td>0.50 ± 0.09</td>
<td>13</td>
<td>18</td>
<td>0.17 ± 0.05</td>
<td>15</td>
<td>0.08 ± 0.03</td>
<td></td>
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<td></td>
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<tr>
<td>Kidney</td>
<td>0.63 ± 0.14</td>
<td>13</td>
<td>18</td>
<td>0.23 ± 0.07</td>
<td>15</td>
<td>0.10 ± 0.04</td>
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<tr>
<td>Lungs</td>
<td>0.82 ± 0.18</td>
<td>13</td>
<td>18</td>
<td>0.33 ± 0.11</td>
<td>15</td>
<td>0.15 ± 0.05</td>
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<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1.96 ± 0.36</td>
<td>13</td>
<td>18</td>
<td>0.73 ± 0.24</td>
<td>15</td>
<td>0.33 ± 0.13</td>
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</table>

* Mean ± SD.
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(16, 39). In addition, radioprotection may also be the result of the ability of IL-1 to induce the production of acute-phase reaction proteins which have free radical-scavenging capabilities (17). IL-1 is also able to stimulate the production of other colony-stimulating factors (40). It is for this reason that we are currently exploring the myeloprotective and recovery possibilities of combining IL-1 with other growth factors (e.g., GM-CSF). Enhanced recovery of myelopoietic cells following 5-fluorouracil treatment has been documented through synergistic interactions of IL-1 and granulocyte colony-stimulating factor (41).

Although we have observed an IL-1-induced, moderate reduction in radioantibody uptake into tumors 24 h after radioantibody administration (Table 1), the effect is temporary and, most importantly, does not affect the therapeutic potential of the radioantibody. One can postulate several reasons to explain this initial reduction in antibody uptake in IL-1-treated animals. IL-1 may affect the hemodynamics of the tumor vasculature and thereby modify the amount of blood delivered to the tumor or the permeability of tumor vessels to large antibody molecules. In one report, it was observed that IL-1 inhibited the action of endothelial growth factor (42). Alternatively, IL-1 may reduce the amount of antigen expressed. Other biological response modifiers, such as interferon and transforming growth factor beta, are able to augment antigen expression (43, 44). These possibilities are currently being evaluated.

The precise dose of cytokine to radioantibody and the timing and sequence of administration of the two agents remain to be determined. It is known, however, that higher colony-forming unit-cellular augmentation is achieved when IL-1 is administered in multiple doses rather than as a single injection (17). IL-1 alone is not tumoricidal at this dose (10^5 units or 333 ng), although higher doses of IL-1 (10–20 μg) have been reported to be cytostatic or cytotoxic both in vitro (45) and in vivo (46). We are currently evaluating the radioantibody distribution and therapeutic efficacy in animals treated with GM-CSF and the effectiveness of IL-1 or GM-CSF at reducing thrombocytopenia, another focus of hematopoietic damage due to irradiation (10, 11).

In summary, it is now possible to administer therapeutic doses of radioantibody in the presence of a cytokine, such as IL-1, with significantly less hematopoietic toxicity. This enables the use of larger and potentially more therapeutic doses of radioantibody which would otherwise not be tolerated.

Acknowledgments

We thank Dr. Peter Lomedico of Hoffmann-LaRoche for providing us with recombinant interleukin 1 and Dr. Steven Gillis of Immunex for providing us with recombinant granulocyte-macrophage colony-stimulating factor.

References

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