ABSTRACT

Radioimmunotherapy with $^{131}$I-labeled monoclonal immunoglobulins was studied using the Rauscher murine erythroleukemia. Tumor-specific monoclonal antibody, nonrelevant monoclonal antibody, $F(ab')_2$ fragments, polyclonal $\gamma$-globulin, and serum albumin were used as carriers of $^{131}$I. Therapeutic effects as measured by the reduction in splenomegaly were seen with all the radiolabeled proteins tested, but not with $^{131}$I-tyrosine. Dose-response curves showed that about 90% reduction in spleen size occurred at 80 $\mu$Ci injected per animal, irrespective of whether specific or nonrelevant monoclonal antibody was used. Therapeutic efficacy was affected by the size of the $^{131}$I-carrier and could be correlated with half-life of carrier protein in vivo. As expected, increase in the serum concentration of circulating antigen decreased the targeting of the tumor-specific monoclonal antibody and also contributed to a shorter half-life for the tumor-specific monoclonal antibody in leukemic animals compared to uninfected controls. This study showed that there was no therapeutic advantage to the use of tumor-specific monoclonal antibody over nonrelevant immunoglobulin as a carrier for $^{131}$I in the treatment of murine erythroleukemia and that, although it was extremely effective, radioimmunotherapy with $^{131}$I was not specific in this system.

INTRODUCTION

New techniques permitting the production of unlimited quantities of monoclonal immunoglobulins of absolute specificity have provided impetus to the goal of using antibodies directed against tumor-specific antigens in cancer therapy (18). The increased specificity of these antibodies and the variety of radionuclides which may now be attached to them have resulted in successful diagnostic tumor imaging with radiolabeled antibodies (13, 15, 35). Nevertheless, many unresolved problems limit the use of monoclonal antibodies in cancer therapy (2, 32). Although some monoclonal antibodies have been shown to be efficacious by either direct cytotoxic action against tumor cells or modulating host immune response (19, 22, 25), exploitation of monoclonal antibodies conjugated to drugs, radioisotopes, or toxins offers the greatest hope of successful clinical application in providing tumor-specific cytotoxic agents of high therapeutic index (32).

Few studies have addressed specifically the role of radiolabeled monoclonal antibodies in the treatment of animal tumors as a means to explore the clinical use of these potentially powerful tools in cancer medicine. Previous studies have utilized polyclonal immunoglobulins or affinity-purified polyclonal immunoglobulins, and thus, the roles of specificity, class, and isotype of the carrier immunoglobulin have not been established (11, 14, 30, 31). The objectives of this study were therefore to evaluate the therapeutic efficacy of radiolabeled tumor-specific and non-relevant monoclonal immunoglobulins, $F(ab')_2$ fragments, polyclonal immunoglobulins, or serum albumin. We examined the influence of the tumor burden and circulating antigen, the specific activity of the carrier proteins, and the effective half-lives of the radiolabeled proteins on the dose-response relationships.

The Rauscher erythroleukemia of mice was chosen for these studies not only because it provides a convenient model with rapid disease progression and readily quantitatable tumor burden, but also because it is an actual disease of mice and not a transplanted tumor system (29). The target organ for the radiolabeled antibody was the spleen, which served in the model as a pseudosolid tumor for quantitation of tumor progression; however, it is well recognized that this organ is part of the reticuloendothelial system, and the disease is a systemic disease which progresses to frank leukemia. We have shown previously that tumor-specific monoclonal antibodies in small doses are therapeutic in this animal model (33). Monoclonal antibody labeled with $^{131}$I showed no increase in potency over unlabeled antibody when given in doses ranging from 0.5 to 5.0 $\mu$Ci/mouse (33). From studies carried out in vitro, however, we have shown that $^{131}$I-labeled monoclonal antibodies can be cytotoxic to these tumor cells at high doses and in a time-dependent manner (38). In this report, we extend these studies to trials in vivo. Mice were treated with $^{131}$I-labeled monoclonal antibodies, polyclonal immunoglobulins, or serum albumin 13 days after initiation of the leukemia when the tumor burden of the animal first becomes clinically apparent (development of palpable splenomegaly). The therapeutic efficacy of the treatments was determined by reduction in the splenomegaly, as well as by the long-term survival of the animals. Hematological profiles of the $^{131}$I-treated and untreated animals were obtained to quantitate the myelosuppression caused by the radiotherapy and the influence of treatment on the development of systemic leukemia in surviving animals. We demonstrate that $^{131}$I-labeled immunoglobulins are therapeutically effective against the leukemia, but that the effects are not immunospecific in this system.

MATERIALS AND METHODS

Rauscher Murine Leukemia Model System. The Rauscher leukemia causes an erythroleukemia characterized by the rapid transformation of erythroid precursor cells, splenomegaly, viremia, and late development of systemic leukemias (3, 4, 7, 29). After virus infection of mice, neoplastic erythroblast in the spleen are visible microscopically within a few days; macroscopic tumor foci are visible on the spleen surface after 6 to 8 days, and 10-fold increases in spleen size may occur in 2 weeks (1, 28, 36). The viral gp70 is expressed in large amounts on the surface

1 This research was supported by National Cancer Institute Contract 1-CP81052.
2 To whom requests for reprints should be addressed.
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of the virus-transformed leukemic cells and was used as the leukemic cell-specific target antigen in these studies.

Monoclonal Antibodies and Other Immunoglobulins. The monoclonal antibodies 103A, an IgG1 reactive with Rauscher murine erythroleukemia cells, and 263D, a control IgG1, were prepared as described previously (33, 35). Monospecific goat anti-Rauscher virus gp70 antibody has also been described previously (37). Bovine IgG (Pentex bovine gamma-globulin; Miles Laboratories, Inc., Elkhart, IN), mouse IgG F(ab')2 fragments (Cappel Laboratories, Westchester, PA), BSA (Sigma Chemical Co., St. Louis, MO) were also used as carrier proteins for the radiotherapy studies.

Purification of Immunoglobulins and iodination. The monoclonal antibodies were purified from ascites fluid by affinity chromatography on Protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) as described (10). The goat IgG anti-Rauscher virus gp70 was purified by 0 to 45% ammonium sulfate precipitation followed by ion-exchange chromatography on DEAE-cellulose (Whatman DE 23). Each of the immunoglobulin preparations and the BSA were assessed to be more than 95% pure based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (37). All of the proteins were iodinated with 125I or 131I by use of chloramine-T as described by Hunter (16), and the reactions were terminated by addition of excess cold tyrosine (17). The labeled proteins were separated from free radioiodines by gel filtration on Sephadex G-50 (Pharmacia Fine Chemicals, Inc.). Specific activities ranged from 2 to 12 μCi/μg.

Targeting of Antibodies to Spleen Cells in Live Animals. The targeting of monoclonal antibodies to spleen cells in vivo was measured as follows. BALB/c mice (Hartan Sprague-Dawley, Indianapolis, IN), 5 to 7 weeks of age, were infected with 250 μl of a 1:90 dilution of a 20% suspension of RVB3 (33). This dose of virus gave about 20 foci on Day 8 as measured by spleen focus-forming assay (1). These mice and uninfected littermates were given i.v. injections of 125I-labeled monoclonal antibody (5 μCi at 7 to 12 μCi/μg). The spleens were removed 1 hr later, and the cells were isolated. Spleen cells were washed 3 times with 10 ml of Dulbecco's phosphate-buffered saline containing 1% BSA as described previously (33, 34). The cells were enumerated in a Coulter Counter (Coulter Electronics, Inc., Hialeah, FL), and the radioactivity in the cells was measured in an LKB gamma counter. The uptake ratio was defined as the cpm bound to 10^6 normal spleen cells divided by the cpm bound to 10^6 leukemic spleen cells divided by the cpm bound to 10^6 normal spleen cells.

Radioimmunotherapy. Groups of 4 mice were infected i.v. with RVB3 virus as described previously (33), and 13 days later, they were treated with either i.v. or i.p. injections of 30 μCi of 125I-labeled immunoglobulins or 131I-labeled serum albumin in 250 μl of isotonic phosphate-buffered saline, pH 7.4. The dose of radioactivity delivered varied from 0 to 160 μCi per mouse. Lugol's solution was administered to all animals in the drinking water from Days -2 to +20 in order to block thyroidal uptake of free radioactive iodine. Mice were sacrificed 7 days (Day 20 after infection), 57 days, and 167 days after treatment, and the spleens were analyzed as described previously (33) and below.

Determination of Half-Life of Radioiodinated Proteins in Vivo. The half-lives of the immunoglobulins of serum were determined by injecting the 125I-labeled proteins i.v. into 4 mice. At various times after injection, the radioactivity was measured in sera obtained by retroorbital bleeding. The measurements of individual mice in each group varied from 10 to 15%, and half-lives were obtained from the linear part of the disappearance curves. The radioactivity in these sera was verified to be attached to the immunoglobulins or BSA by either trichloroacetic acid precipitation or polyacrylamide gel electrophoresis, followed by autoradiography.

Determination of gp70 Concentration in Serum. The concentration of gp70 in serum of mice was measured by solid-phase radioimmunoassay in polystyrene microtiter wells (Falcon, Oxnard, CA) as described previously (23). Briefly, the microtiter wells were incubated at 37° overnight with 50 μl of isotonic phosphate-buffered saline, pH 7.0, containing 1 μg of monoclonal antibody 103A. The remaining protein-binding sites in the well were then saturated by the addition of 200 μl of phosphate-buffered saline [20 mM phosphate (pH 7.4):150 mM NaCl] containing 5% calf serum, 0.1% Triton X-100, and 0.02% NaN3, and the wells were incubated for an additional 90 min at 37°. After removal of this buffer, 50 μl of the test serum samples or purified gp70 diluted in normal mouse serum were added, and the wells were incubated for 1 hr at 37°. After 3 washings with the above buffer, 50 μl containing 500 ng of purified goat IgG anti-Rauscher gp70 were added, the wells were again washed 3 times, and bound antibodies were quantitated by addition of 50 μl containing 5 ng of 125I-labeled pig IgG directed against goat IgG. Unbound antibody was again removed by washing. After incubation with 80 μl of 2 μM NaOH for 15 min at 65°, the solubilized proteins were transferred to glass tubes, and the radioactivity was measured in an LKB gamma counter.

Hematological and Histological Profiles. Blood for analysis was obtained by puncture of the retroorbital venous plexus with a heparinized capillary tube. RBC and WBC were counted by the use of a Coulter Counter. Packed cell volumes were measured directly after centrifugation in a microhematocrit centrifuge. Platelets were counted with the use of a microhemocytometer. Peripheral blood smears were stained with either Wright's mixture or Wright's Geimsa stain. Differential counts were based on 100 cells counted. Bone marrow imprints were prepared from the femur and stained with Wright's Geimsa.

Touch preparations and thin sections of the infected spleens were stained with either Wright's Geimsa or hematoxylin:eosin.

RESULTS

Tumor Targeting of Monoclonal Antibody In Vivo. The specific targeting of 103A IgG to leukemic spleen cells of the RVB3-infected mice has been demonstrated previously (33). Targeting of 103A IgG to leukemic spleen cells occurred rapidly, followed by a time-dependent decrease in the tumor:normal cell uptake ratio of the antibody, presumably due to tumor antigen shedding or virus budding, with concomitant displacement of and catabolism of the bound antibody (34). The present studies were designed to evaluate the influence of circulating antigen on tumor targeting. At various times after RVB3 virus infection, the concentration of gp70 was measured in serum samples from leukemic and uninfected mice. These mice were then injected with 125I-labeled 103A antibody. After 1 hr, the animals were killed, their spleens were removed and weighed, and the amount of radioactivity bound to the isolated spleen cells was determined. The concentration of gp70 in serum increased with time after RVB3 virus infection as measured on Days 3 through 26, and there was a logarithmic increase in spleen size over this same period. Uninfected mice never contained more than 20 ng (background) of gp70 per ml of serum. Similar values were found for the treated leukemic mice up to 6 days. By Days 13, 20, and 26, however, the concentration of gp70 had risen to 3.5, 6.5, and 10 μg/ml, respectively. The concentration of gp70 in the serum correlated with the spleen size and number of isolated spleen cells; a linear regression analysis of 20 data points has a correlation coefficient of 0.85. Since 103A IgG binds equally well in vitro to viral gp70 and to isolated RVB3-infected splenocytes (34), it would be expected that, as the serum concentration of gp70 increased, there would be a corresponding decrease in the amount of 103A IgG targeting to spleen cells in vivo. This correlation was observed. Targeting of 5 μg of 125I-labeled antibody decreased sharply as the concentration of gp70 per ml of serum rose (Chart 1).

Immunotherapy with 103A IgG. In a previous study, we have shown dramatic therapeutic effects from the administration of
"I IMMUNOTHERAPY OF MURINE ERYTHROLEUKEMIA

Chart 1. Correlation between the binding of "I-103A IgG to leukemia spleen cells and the concentration of circulating antigen, gp70. The assays were conducted as described in "Materials and Methods" on the following days after virus infection: A, Day 13; D, Day 20; and O, Day 26.

Chart 2. Dose-response curve for radioimmunotherapy of Rauscher erythroleukemia with "I-103A IgG, measured as the percentage of reduction from control spleen weight. One hundred % reduction was a normal spleen, 100 to 150 mg; 0% reduction was 1.0 to 1.5 g. Points, mean of 4 to 7 mice; bars, S.D. A dose of 30 µg "I-103A IgG was injected into each animal at 13 days after virus infection. Specific activities ranged from 0 to 5.3 µCi/µg.

unlabeled 103A IgG to mice within 3 days of RVB3 virus infection (33). At the later times examined in the current study, however, increasing amounts of 103A were required for therapeutic effect. An 80% reduction in spleen size was observed with only 2.5 µg of 103A IgG at 3 days postinfection. In contrast, at 13 days postinfection, 80 µg of 103A IgG per mouse were not therapeutic. The therapeutic efficacy of unlabeled 103A IgG therefore decreased with the duration of infection as the tumor burden increased and as the serum concentration of gp70 rose. Decreasing therapeutic efficacy of 103A IgG also correlated with decreasing tumor targeting.

Radioimmunotherapy with "I-103A IgG. A fixed dose of "I-103A (30 µg) was used in the radiotherapy experiments, since it had been established that there was no therapeutic effect from this dose of unlabeled 103A at 13 days postinfection. The specific activity of the carrier protein was varied between 0 and 5.33 µCi/µg to deliver doses ranging from 0 to 160 µCi per animal. At doses of 160 µCi per animal, there was a dramatic reduction in spleen size, establishing the therapeutic efficacy of "I-103A IgG. The dose-response curve (Chart 2) showed that no significant reduction in spleen size occurred with doses less than 45 µCi per animal. Delivery of "I conjugated to tyrosine in equivalent doses (i.e., 0 to 160 µCi per animal) had no therapeutic effect. The presence or absence of Lugol’s solution in the drinking water of the treated animals had no effect on spleen size.

Radiotherapy with Other Immunoglobulins. In order to determine whether antibody specificity contributed to the therapeutic effects observed with "I-103A IgG, 3 other nonspecific radioiodinated immunoglobulins were tested. Reduction in spleen size at 13 days postinfection was observed with each of these immunoglobulins. A direct comparison of the dose-response curves for "I-103A IgG and nonrelevant monoclonal antibody "I-263D IgG is shown in Chart 3A. When the specific activity was more than 1.5 µCi/µg, there was no statistically significant difference between these monoclonal antibodies, but at lower values, the nonrelevant monoclonal antibody appeared to be more effective. The activities of heterologous versus homologous antibodies and polyclonal versus monoclonal antibodies were compared using "I-labeled polyvalent bovine IgG and "I-263D IgG. There was no statistically significant difference between these immunoglobulins over the range of specific activities tested (0 to 5.3 µCi/µg) (Chart 3, A and B). The roles of Fc receptor and size of carrier protein were evaluated by testing "I-labeled polyvalent mouse F(ab')2 and "I-BSA, respectively. F(ab')2 was more effective than BSA, but both were less effective than polyvalent IgG (Chart 3B).
Half-Lives of the Immunoglobulins. Characterization of the pharmacokinetics of radiolabeled antibodies should be a prerequisite for their clinical use as drugs. Semilogarithmic plots of the clearance curves were biphasic. Half-lives determined from the slopes of the slower component are listed in Table 1. The order of therapeutic efficacy [IgG > F(ab'), IgG (polyclonal) > BSA] correlated directly with the half-lives of the radioiodinated proteins in vivo. The single statistically significant difference in the half-lives of the radioiodinated proteins between leukemic and control mice was observed with 103A IgG (Table 1). The rapidity of clearance of the specific antibody in the leukemic animals compared with a slower pattern of clearance in control animals is apparent in Chart 4. In contrast, for 263D IgG and F(ab')2, there was no observable difference in clearance rates between leukemic and control mice (Chart 4).

Temporal Response to Radiotherapy. Radioiodinated immunoglobulin caused a reduction in spleen size in both leukemic and control mice, but the proportionate reduction was greater in the leukemic spleens. The mean weight of leukemic spleens at 13 days postinfection was 330 mg compared with 120 mg for control spleens. Treatment with 160 μCi of 131I-IgG caused a reduction in the weights of leukemic and control spleens to mean values of 50 and 35 mg, respectively. This response to radiation was transient, and regrowth of all treated spleens occurred with time. Chart 5 illustrates this initial response to therapy with 131I-IgG and the subsequent regrowth to the original weights of both the RVB3-infected and control spleens. Over the same 20-day period, the untreated leukemic spleens grew exponentially. The treated leukemic spleens initially decreased in size and then regrew. However, a dose response to the therapy was still apparent at Days 57 and 167 in the surviving animals (Table 2). No significant difference existed in the weights of the treated and untreated control spleens at long-term follow-up.

Histological Studies. At Day 20, RVB3-infected spleens were very large with spongy tissue containing blood-filled cysts. Thin sections showed diffuse erythroblastic proliferation, with progressive atrophy of white pulp and rupture of trabeculae (3, 8, 29). Gross examination at Day 57 of spleens from treated mice showed that, with the higher doses of radiation, the spleens were smaller and firmer and had a grayish-red appearance. Thin sections of these irradiated spleens showed areas of recognizable red and white pulp, but interspersed between normal structures were clones of leukemic-appearing erythroblasts. Histological examination of the treated control spleens at 57 days showed normal architecture with no evidence of radiation damage.

Hematological Consequence of Virus Infection and Response to Radiotherapy. In BALB/c mice, the disease caused by Rauscher virus is characterized by 2 distinct phases: splenic erythroblastosis followed later by a generalized leukemic process (3-5, 7). The majority of animals died during the early phase, so that relatively few animals survived to manifest generalized leukemia. The hematological profile of the surviving animals showed a gradual decrease in hematocrit readings from an average of

<table>
<thead>
<tr>
<th>Protein</th>
<th>RVB3 infected</th>
<th>Controls</th>
<th>p value of leukemic versus control</th>
</tr>
</thead>
<tbody>
<tr>
<td>103A IgG</td>
<td>47.9</td>
<td>100.1</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>263D IgG</td>
<td>80.9</td>
<td>105.4</td>
<td>NS</td>
</tr>
<tr>
<td>IgG (polyclonal)</td>
<td>56.5</td>
<td>66.5</td>
<td>NS</td>
</tr>
<tr>
<td>F(ab')2 (polyclonal)</td>
<td>40.5</td>
<td>50.8</td>
<td>NS</td>
</tr>
<tr>
<td>BSA</td>
<td>32.2</td>
<td>33.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Two-tailed t analysis of the linear regression slopes for the clearance of each protein in leukemic and control mice.

# NS, not statistically significant.

Chart 4. Clearance of 125I-labeled immunoglobulins from the serum of erythroleukemic and control mice. The assays were conducted as described in "Materials and Methods." Closed symbols represent leukemic animals; open symbols refer to control animals. Points, mean of 4 to 8 mice: ○ and ●, 103A; △ and □, 263D; □ and ■, F(ab')2.

Chart 5. Progression of splenomegaly with time following i.v. injection of Rauscher leukemia virus into mice and response to nonspecific radioimmunotherapy. Thirty μg of bovine 131I-IgG (specific activity, 5.3 μCi/μg) were administered on Day 13 to leukemic and control mice. The assays were conducted as described in "Materials and Methods." Points, mean of 4 to 8 mice; S.E. s were ±12.5%. ○, leukemic mice, untreated; □, leukemic mice treated with 131I-IgG; ---, control mice; ●, control mice treated with 131I-IgG.
54 to 35%, a gradual decrease in platelet count from a mean of 1.1 x 10^9/cu mm to platelet counts as low as 1.8 x 10^5/cu mm, and a corresponding increase in total leukocyte count from an average of 7 x 10^3/cu mm to 9 x 10^4/cu mm (around 50 days). Peripheral smears showed a classic macrocytic anemia with 60 to 70% mononuclear cells and many smudge cells present.

Frankly leukemic erythroblasts were occasionally seen in the peripheral blood of animals as a late manifestation of disease. An increase in nucleated RBC, up to 30% of the total leukocyte count, was also found at this stage. The bone marrows of these mice were hypercellular and contained sheets of classic erythroleukemic proliferation with marked reduction of normal elements.

Administration of ^131I-labeled IgG to all mice caused a rapid decrease in peripheral leukocyte counts, primarily due to loss of circulating lymphocytes (Chart 6). This appeared to be a systemic effect of the ^131I analogous to total body irradiation (24, 39). Less dramatic decreases in RBC and platelet counts also were observed. In treated normal mice, there was complete recovery of all peripheral blood elements, including RBC and platelets, at 3 to 4 weeks. The nadir in the leukocyte counts was found between 5 and 11 days, the level depending on the dose of radioactivity delivered. A maximum of 160 μCi/mouse was chosen, because this was found to decrease the leukocyte count in uninjected mice to the aplastic threshold of 100/cu mm.

Protein-conjugated ^131I-IgG (specific activity, 5.3 μCi/μg) were administered to leukemic and to control mice. The analyses were performed as described in "Materials and Methods." Points, mean of 4 to 8 mice; S.E.s were ±10%. Closed symbols represent the animals treated with ^131I-IgG; open symbols refer to the untreated mice. 

Compared to untreated leukemic mice which had all died by Day 60, Animals that received larger doses of radioisotopes survived proportionately longer, and the mice treated with 160 μCi, the highest dose tested, survived up to 190 days (Chart 7).

**DISCUSSION**

Previous studies in this (33) and other systems (11) have shown tumor-specific therapeutic effects of unlabeled monoclonal antibodies in the therapy of animal tumors. In the present studies, designed to examine the effect of ^131I-labeled immunoglobulins, we found no advantage to treatment with tumor-specific antibody as a carrier for ^131I; similar therapeutic effects were seen when equivalent doses of radioisotope were conjugated to nonrelevant monoclonal antibody or to polyvalent heterologous IgG. Despite this nonspecificity, protein-conjugated ^131I was therapeutically effective. In addition, since unlabeled antibody was not therapeutically effective at these doses and

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**Table 2**

<table>
<thead>
<tr>
<th>Mouse group</th>
<th>Time after treatment (days)</th>
<th>Dose of ^131I-IgG (μCi/mouse)</th>
<th>Spleen wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>0</td>
<td>120 ± 20</td>
</tr>
<tr>
<td>RVB3 infected</td>
<td>7</td>
<td>160</td>
<td>35 ± 15</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>160</td>
<td>120 ± 15</td>
</tr>
<tr>
<td></td>
<td>167</td>
<td>160</td>
<td>100 ± 20</td>
</tr>
<tr>
<td>RVB3 infected</td>
<td>7</td>
<td>0</td>
<td>910 ± 250</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>160</td>
<td>55 ± 30</td>
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<td></td>
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<td>40</td>
<td>1020 ± 205</td>
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<td>57</td>
<td>80</td>
<td>230 ± 80</td>
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<td>160</td>
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<td></td>
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<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>167</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
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<td>40</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>167</td>
<td>80</td>
<td>2025 ± 310</td>
</tr>
<tr>
<td></td>
<td>167</td>
<td>160</td>
<td>1675 ± 150</td>
</tr>
</tbody>
</table>

*Groups of 4 to 8 mice were treated with ^131I-IgG at Day 13 after viral infection, and response to therapy was determined on the days indicated. Body weights of all animals were recorded and ranged between 20.8 and 22.9 g over the time period studied.

<sup>a</sup> Mean ± S.D.

<sup>b</sup> No surviving animals in the groups indicated.
tumor burdens, the tumoricidal results seen in these studies can be attributed solely to the nonspecific delivery of $^{131}$I to the tumor. Similar nonspecific killing has also been observed in this system in vitro (38). The size of the carrier was important, as this was a major determinant of the half-life of the $^{131}$I in vivo. As expected, the increase in serum concentration of tumor antigen correlated with decrease in tumor targeting and probably contributed to the shorter half-life of the specific antibody in leukemic compared with control mice observed here and previously (34). No differences in either the antibody half-life or therapeutic efficacy of the IgG were seen by changing the route of injection from i.v. to i.p. However, it will be important to investigate other routes of administration of immunoglobulins in the light of the work of Weinstein et al. (40, 41), who showed that, after s.c. injection, monoclonal antibodies entered local lymphatic capillaries and reached regional nodes where they were able to bind to tumor metastases. These authors suggested that the lymphatic route may minimize binding to circulating tumor antigens and to cross-reactive antigens in normal tissues.

Goldenberg et al. (14) have studied the effects of $^{131}$I-anti-carinoembryonic antigen antibodies directed against human colonic carcinoma GW-39 implanted in hamster cheek pouch. At doses of 1 mCi and greater, there was a marked growth inhibition of GW-39 tumors and an increase in survival time in the tumor-bearing animals. At doses of 2 mCi, there was no significant difference in the therapeutic efficacy of $^{131}$I-anti-carinoembrionic antigen immunoglobulins compared with radiolabeled nonspecific IgG, prompting the conclusion that, with high doses of radiolabeled antibodies, nonspecific antitumor effects can be achieved because of the increased accretion of IgG in tumors (14). In contrast, others (12) have observed selective killing by $^{131}$I-labeled polyclonal antibody in an i.p. murine lymphoma model. However, these studies markedly differed in the timing and quantity of administered antibody.

Survival of treated leukemic mice past an early phase when death would have otherwise occurred due to erythroblastosis, advanced splenomegaly, splenic rupture, and hemorrhage probably accounted for the favorable survival rates in treated mice. Nevertheless, these mice eventually developed systemic leukemia and succumbed to the disease. The longest survivors (up to 190 days) were the mice treated with the highest doses of radiolabeled immunoglobulin. It is tempting to speculate that actual cures could be achieved with timed-sequential radioimmunotherapy treatments in an analogous manner to the use of chemotherapy regimens for the treatment of human leukemia (6).

For the findings of this study to have relevance to radioimmunotherapy in humans requires that the administered dose of $^{131}$I be clinically appropriate. The extrapolation to man was made by considering both the total absorbed dose in the spleen and the concomitant total body irradiation. These parameters were calculated, using the analyses of Leichner and coworkers (20, 30, 31). The total absorbed dose in any organ is proportional to the product of the initial concentration and effective half-life of the isotope in that organ (20). It has been shown previously that there is no selective uptake of nonrelevant immunoglobulins in mouse spleen and that their half-life in spleen and blood is equal (34). It is therefore possible to use this formula (20) to calculate the total absorbed dose of radiation in this model for $^{131}$I-polyvalent bovine IgG.

The effective half-life was calculated from the measured biological half-life (Table 1) and the physical half-life of $^{131}$I. This isotope decays by $\gamma$ and $\beta$ transitions with production of internal conversion electrons, Auger electrons, and low-energy X-rays. The majority of the dose deposited in a small organ is from the nonpenetrating components, for which the accumulated radiation is $0.41 \text{ g-rad/mCi-hr}$ (20). Using this formulation, the calculated mean absorbed doses to the spleen and to the total body of a 25-g mouse treated with 160 $\mu$Ci of $^{131}$I-polyvalent bovine IgG were 1800 and 165 rads, respectively. The values of both dose delivered to the tumor and total body irradiation with subsequent reversible myelosuppression are in good agreement with those reported in clinical radioimmunotherapy studies (9, 20). The clinical use of radioimmunotherapy has been restricted to the treatment of hepatomas, lymphomas, and melanomas, and in some cases, dramatic therapeutic effects have been observed (2, 20, 21, 26, 27). The dose-limiting toxicity appears to be myelosuppression (9). In clinical trials on hepatoma, a polyclonal heterologous IgG with approximately 20% antigen specificity was used as the carrier immunoglobulin (26, 27). Specific monoclonal antibody was found not to be therapeutically effective because of its rapid catabolism and short half-life in serum. The results here and previously also show that the specific monoclonal antibody was cleared more rapidly than the nonrelevant antibody in tumor-bearing animals. We interpret this shorter half-life of the specific monoclonal antibody as direct evidence that specific interaction is occurring in vivo, and the finding of free iodine in the gut of leukemic animals is secondary to tumor-mediated catabolism of the bound monoclonal antibody (34). The current study suggests that $^{131}$I radioimmunotherapy may benefit human tumors involving the reticuloendothelial system, which include leukemias and some lymphomas. When the tumor burden is large, the use of tumor-specific monoclonal antibody may offer no therapeutic advantage over polyvalent $\gamma$-globulin as a non-specific carrier for $^{131}$I. After cytoreduction has occurred, however, when the goal of therapy is to eradicate micrometastases, tumor-specific monoclonal antibodies could be used, but these must act quickly upon targeting. In this regard, the use of monoclonal antibodies conjugated to radionuclides with high linear energy transfer, such as the $\alpha$-emitting radionuclides, may provide the highest therapeutic index (32, 34).

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Specificity, Efficacy, and Toxicity of Radioimmunotherapy in Erythroleukemic Mice

William R. Redwood, Timothy D. Tom and Mette Strand


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