Experimental Radioimmunotherapy of a Xenografted Human Colonic Tumor (GW-39) Producing Carcinoembryonic Antigen

David M. Goldenberg, Shai A. Gaffar, Sidney J. Bennett, and J. Larry Beach

MATERIALS AND METHODS

Tumor Model. The GW-39 tumor system is a human colonic carcinoma serially transplanted in various body sites of unconditioned adult golden hamsters (16). This tumor has been found to produce large quantities of CEA (10). It has also been shown that GW-39 tumors are not very responsive to a number of anticancer drugs and to external X-irradiation (8), thus resembling the behavior of human colonic cancer. In the experiments performed, freshly excised GW-39 tumors were made into a 10% suspension in 0.01 M phosphate buffer (pH 7.4) containing 0.15 M NaCl by means of a hand-driven glass homogenizer. Each cheek pouch of the hamsters received 0.5 ml of this tumor cell suspension. Female hamsters weighing 100 g were randomly divided in groups of 5 to 9 and were caged individually.

Tumor growth rate was determined by measuring the size of each tumor at different intervals after transplantation, beginning on Day 4. The hamsters were anesthetized with methoxyflurane (Metofane), and the cheek pouches were everted to permit measurement of tumor size in 3 dimensions with a small caliper. Tumor size was determined by multiplying length \times width \times thickness in mm. This product served as a baseline for determining increase in tumor size with time, as we have described earlier (8). Changes in body weight were also recorded at regular intervals. Animals were also sacrificed at regular intervals for the determination of radioactivity in the tumors and various tissues and for recording tumor and organ weights. Blood and tissue levels of radioactivity were determined by exsanguinating the anesthetized animals by cardiac puncture and counting 1.0-ml aliquots of unclotted blood and the removed intact organs in a well-type scintillation counter. The organs were not perfused before or after removal from the animal. The tissues were weighed, and the organ radioactivity was computed on a per g basis. Thereafter, the amount of radioactivity in cpm/g was calculated per tissue (specific count rate) and as a percentage of the injected dose, compensating for the decay of the isotope.

Radioiodinated Immunoglobulins. The IgG fraction of antisera to CEA, obtained from a goat (No. 1030) immunized with purified CEA isolated according to Newman et al. (25), was prepared by automated affinity chromatography, as described elsewhere (15, 30). Its purity was confirmed by electrophoresis and immunodiffusion, and the anti-CEA immunoreactivity was established by immunodiffusion and a modification of the Roche CEA radioassay. The titer of the antibody in the radioimmunoassay, at half-maximum binding, was 2 \times 10^6.

Both CEA antibody IgG and normal goat IgG (Lot 15; Miles Laboratories, Inc., Kankakee, Ill.) were radiolabeled with 131I by the chloramine T method of Greenwood et al. (17), as modified by McConahay and Dixon (23). The radiolabeled IgG preparations were separated from unbound radionuclide by filtration on Sephadex G-25 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and then collected in 1% Oixon (23). The radiolabeled IgG preparations were separated from unbound radionuclide by filtration on Sephadex G-200. The specific activity ranged from 7.4 to 25.5 Ci/g without affecting antibody immunoactivity, as measured by binding to a CEA affinity column. The radioantibody contained greater than 90% native IgG and showed 76% immunoactivity on a CEA affinity column.

Experimental Design. The radiolabeled globulin prepartions were administered intracardially as a single dose to hamsters bearing GW-39 cheek pouch tumors transplanted earlier. Three dose levels of radioactivity were applied, 0.5, 1.0, and 2.0 mCi, representing IgG quantities of 25 to 68, 68 to 100, and 82 to 105 \mu g, respectively. In addition to plotting tumor size changes with time, survival time for the animals was also noted.
Radiation-absorbed Dose Calculations. Absorbed dose rates and cumulative doses for tumor and selected organs were estimated from the mean specific activities (in $\mu$Ci/g) of those organs, measured on Days 1, 3, 5, and 20 postinjection. We selected the 7 most prominent $\gamma$ and electron emissions from $^{131}I$, estimated the $\gamma$-absorbed fractions, assuming a spherical configuration, and used an absorbed fraction of unity for the $\beta$ components. Dose rate calculations were based principally on data and classical techniques found in the Medical Internal Radiation Dose documents (3), for the general case.

In the Medical Internal Radiation Dose formulation, the dose rate ($R$) and cumulative dose ($D$) are given, respectively, by:

$$ R(v \rightarrow r) = \frac{\Delta}{m_a} \sum \Delta \Phi_i (v \rightarrow r), \text{ in rads/hr}; $$

$$ D(v \rightarrow r) = \frac{\Delta}{m_a} \sum \Delta \Phi_i (v \rightarrow r), \text{ in rads}; $$

where $v$ is the target volume and $r$ is the source volume. The Medical Internal Radiation Dose "S" values are of course only appropriate for human organ sizes and configurations. In our calculations, we have assumed that $v = r$ for both the $\beta$ and $\gamma$ components, as discussed below. The equilibrium dose rates $\Delta$ (in g-rad/$\mu$Ci-hr) are taken from the Medical Internal Radiation Dose tables. Cumulative specific activities ($A/m_a$), in $\mu$Ci-hr/g, are calculated from

$$ \frac{A}{m_a} = \int_0^t \frac{A(t)}{m_a} dt, $$

where $A(t)$ is determined by linear interpolation of the data measured on Days 1, 3, 5, and 20. The absorbed fractions $\Phi_i$ are set equal to 1.0 for the $\beta$ components and are based on $\mu_b$ (the linear absorption coefficient) and $r$ (the mean organ radius) for the photon components (21). $\Phi (\mu_b, r)$ was estimated to be 0.013, assuming a uniform distribution in a 1.3-g mass.

There was no effort to include individual assessments of organ size, shape, or density, since the $\gamma$-absorbed fraction does not change significantly with size for these energies. We have also assumed a uniform distribution of $^{131}I$ throughout each tumor or organ, at least over regions having dimensions larger than the mean $\beta$ ranges (0.1 to 0.7 mm) (21). It was further assumed that each of the organs examined was larger than these dimensions or that the concentrations in the immediately adjacent tissue is nearly equal to that of the organ in question. Practically speaking, these assumptions would result in a small overestimation of the tumor dose for tumors less than 1 to 2 mm in diameter, which was not the case in these experiments. We made the assumption that the measured activity in resected organs includes activity of $^{131}I$ contained in blood within each organ in roughly the same proportions that exist in the live animal and that the calculated doses therefore reflect the dose in the living animal. The actual organ doses may be slightly higher for the first few days, however, depending on the fractional blood volume in each organ in vivo.

These data ignore the effects of the elevated and rapidly changing dose rates during the first few hr postinjection. The most likely effect of these high dose rates, however, is to elevate the whole-body dose and thereby reduce slightly the tumor/nontumor dose ratio.

RESULTS

Since change in tumor size with time was used to determine the antitumor effects of the radiolabeled preparations, we first evaluated whether tumor size indeed reflected tumor mass or weight. The advantage of using tumors transplanted to the hamster cheek pouch is that they can be everted easily and repeatedly to measure the dimensions of the tumors. A good correlation between increases in tumor size and weight was found. Although tumors can vary in size from one cheek to another, the relative increment in tumor size with time appears to be a fairly constant function, as was reported earlier (8). Thus, tumor inhibition could be assessed by evaluating changes in the mean increase of tumor size with time.

The antitumor effects of a single dose of 0.5 $m$Ci of $^{131}$I-labeled anti-CEA IgG are shown in Chart 1 and indicates that this had little effect on the tumors at the different measurement points following administration, although a slight prolongation of survival of the radioantibody-treated group over the other groups was observed (Chart 1, insert). The suggestion of unlabeled antibody to CEA having a therapeutic effect on the tumor was not confirmed in a subsequent experiment (Chart 3). It was thus concluded that the radiation dose administered in this manner was insufficient to retard the growth rate of the tumor. However, raising the dose administered to 1.0 $m$Ci did result in evidence of therapeutic efficacy (Chart 2), particularly in the animals receiving radioactive antibody or normal IgG labeled with $^{131}$I. The radioactive antibody appeared to be more effective (70% mean tumor growth inhibition on Day 16 posttherapy) than the radioactive normal goat IgG preparation (53% mean tumor growth inhibition) given at the same dose. These differences are more pronounced when survival curves of the groups are compared (Chart 2, insert). The radioactive anti-CEA IgG group showed a survival of 84% at more than 50 days, whereas the next closest group had all animals dead by 30 days posttherapy. However, the surviving animals did have cheek pouch tumors. During a 20-day observation period, tumor-bearing hamsters showed a mean weight loss of almost 5%, whereas the groups receiving radiiodinated anti-CEA IgG or normal IgG experienced a 20% loss, the nadir being at about 12 days. The animals usually expired because of local expansive tumor growth resulting in inanition.
In order to evaluate the antitumor effects of 1 mCi of 131I-labeled anti-CEA IgG as compared to the same radioactive dose of normal goat IgG, additional groups of 8 to 9 hamsters bearing GW-39 tumors in both cheek pouches were studied and again showed the advantage of the radiolabeled specific antibody preparation over the normal goat IgG in inhibiting the growth of GW-39 tumors, the differences being significant (p < 0.001; Student's t test) on the 3 last observation times, 13, 16, and 20 days following the single injection of the radioactive immunoglobulin preparations.

At a radiation dose of 2 mCi/hamster, the groups showing the best therapeutic effects were those receiving the radiolabeled immunoglobulin preparations (Chart 3). Indeed, at this dose, no difference could be seen between the antibody and normal IgG preparations, either in terms of retardation of tumor growth or increase in survival time. The group receiving cold antibody CEA did not show any evidence of antitumor effects. The percentage of injected radioactivity recovered on various observation days for the antibody and control groups of animals, per g of tissue, is recorded in Table 1. The tumors receiving the radiolabeled antibody show an increase in the percentage of radioactivity injected present per g at up to Day 5 and then a decrease by Day 20. All other organs, however, and the results with animals receiving the control IgG preparation indicate a gradual loss of the radioactivity administered. It is interesting that the highest percentage of radioactivity localized in the tumors was found to be 2.57% of the injected dose, on Day 3.

The change in specific activities (µCi/g tissue) for the 2 experimental groups on various days following treatment is displayed in Chart 4. A marked drop in blood levels in the treated, in contrast to the control, animals is seen. As is to be expected, the group receiving specific radioactive antibody maintained the highest specific activity levels in the tumor throughout the duration of the experiment, whereas the specific activities in the other organs did not differ ostensibly between animals receiving specific antibody or normal IgG preparations. This difference between specific activity in the tumors receiving specific antibody and those receiving normal goat IgG was significant at a p value of less than 0.01 in Student's t test. It is noteworthy that, in the group receiving 131I-labeled anti-CEA IgG, the blood levels fall more rapidly than the physical half-life during the first 5 days, while the tumor levels remain fairly constant despite physical decay of the isotope. This implies an increased tumor uptake in spite of tumor growth and biological elimination of the radioactive preparation.

The tumor/nontumor ratios for 131I-labeled anti-CEA IgG and 131I-labeled normal goat IgG on the various observation days posttherapy are shown in Table 2. These data were derived by comparing the radioactivity (µCi/g) of tumor to that of the various organs of the animals. In the case of blood, the radioactivity/1 ml was used as a basis of comparison. It can be seen that the tumor/nontumor ratios increased from Day 1 to Day 20 for the group receiving the specific antibody, whereas much smaller changes were observed for the control normal IgG animals. The increase on Day 20 for the group receiving the anti-CEA IgG is apparently due to the very low specific activities in the various normal organs relative to tumor, at this time, as is also shown in the data of Tables 1 and 3. On most of the observation days, the highest ratios were for the tumors in comparison to the liver and peritoneal muscle for the antibody
Table 1

Distribution of radiolabeled IgG in tissues (1-mCi dose)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Day 1 Anti-CEA IgG</th>
<th>Day 1 Normal IgG</th>
<th>Day 3 Anti-CEA IgG</th>
<th>Day 3 Normal IgG</th>
<th>Day 5 Anti-CEA IgG</th>
<th>Day 5 Normal IgG</th>
<th>Day 20 Anti-CEA IgG</th>
<th>Day 20 Normal IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>1.85</td>
<td>0.98</td>
<td>2.57</td>
<td>0.93</td>
<td>2.48</td>
<td>0.60</td>
<td>0.30</td>
<td>0.06</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.31</td>
<td>0.85</td>
<td>0.49</td>
<td>0.86</td>
<td>0.89</td>
<td>0.58</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Heart</td>
<td>0.95</td>
<td>0.83</td>
<td>1.02</td>
<td>0.82</td>
<td>0.37</td>
<td>0.55</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Liver</td>
<td>0.64</td>
<td>0.48</td>
<td>0.31</td>
<td>0.45</td>
<td>0.31</td>
<td>0.38</td>
<td>0.005</td>
<td>0.02</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.59</td>
<td>0.38</td>
<td>0.30</td>
<td>0.45</td>
<td>0.31</td>
<td>0.29</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.00</td>
<td>0.55</td>
<td>0.31</td>
<td>0.52</td>
<td>0.32</td>
<td>0.33</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Bladder</td>
<td>1.87</td>
<td>0.98</td>
<td>0.74</td>
<td>1.06</td>
<td>0.61</td>
<td>0.59</td>
<td>0.014</td>
<td>0.06</td>
</tr>
<tr>
<td>Peritoneal muscle</td>
<td>0.76</td>
<td>0.38</td>
<td>0.27</td>
<td>0.35</td>
<td>0.30</td>
<td>0.28</td>
<td>0.005</td>
<td>0.02</td>
</tr>
<tr>
<td>Blood (1 ml)</td>
<td>4.40</td>
<td>2.57</td>
<td>1.19</td>
<td>4.22</td>
<td>1.02</td>
<td>3.46</td>
<td>0.016</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Chart 4. Distribution of specific activity of 1 mCi $^{131}$I administered as a conjugate with anti-CEA IgG or normal IgG to hamsters bearing cheek pouch grafts of GW-39 tumor. A, physical decay; D, tumor; O, blood; •, bone marrow; X, urinary bladder; •, brain; •, mean for lung, liver, kidney, spleen, heart, and peritoneal muscle.

At 20 days posttreatment, the mean cumulative dose of radiation of the tumors was calculated at 1325 rads for the group receiving specific antibody and 411 rads for the group receiving normal IgG. There were not any significant differences noted between these 2 groups for the other organs examined. In the animals treated with specific radioantibody, the mean tumor cumulative dose is a multiple of that calculated for the other organs, ranging from a high of 6.9 for the liver to a low of 2.8 for the urinary bladder. The tumor/nontumor cumulative dose ratios were not increased as much in the group receiving normal radioglobulin (Table 3).

An additional group of 5 hamsters was followed for 40 days postinjection of the radioantibody; the animals were submitted to external NaI (T1) photon counting. Whole-body activity was determined by unshielded counting at 50 cm, and total tumor activity was obtained by shielding all but the everted tumor-bearing cheek pouch and subtracting the background activity obtained with the cheek pouch also shielded. These data indicated an increased uptake of radioactivity in the tumors up to at least Day 25 and a slower release from the whole body at up to Day 40 posttreatment, when the experiment was concluded. The effective half-life leveled off at $T_{1/2} = 6$ days beyond 25 days postinjection. The effective half-life was found to be approximately $T_{1/2} = 15$ days (i.e., active accretion) up to Day 25. If this qualitative information is included in the calculations made above, the estimated tumor dose would increase by 275 rads to 1600 rads. Future experiments will need to determine these long-term effects.

**DISCUSSION**

The general failure of drugs and radiation to control disseminated cancer is a consequence of their inability to discriminate between normal and malignant cells. The immunological approach to cancer treatment has thus been an attractive concept and can be traced back to 1895, when Hericourt and Richet...
have reported success in using therapeutic doses of radioactive antibodies to ferritin or to CEA in the treatment of hepatic cancers. The experiments undertaken here were designed to substantiate the therapeutic action of radioactive CEA antibodies in a CEA-producing human colonic carcinoma transplantation model. We appreciate that this tumor system has certain disadvantages as a study model for human colonic cancer, since we are dealing with a species difference between tumor and host and with an animal that does not have CEA in its tissues or blood, as is the case in humans. Nevertheless, since goat antibodies to CEA can localize in tumors in humans despite the slightly increased tumor accretion of normal IgG is respon-

Our experimental studies have shown previously that antibodies to the CEA of Gold and Freedman (7) can localize selectively in GW-39 human colonic carcinomas propagated serially in hamsters (14, 30, 31). At low doses of radiation, antibodies labeled with \(^{125}\)I could be used for external tumor detection by scintigraphic methods (14). These studies served as a basis for subsequent clinical trials of cancer radioimmunodetection with radioactive CEA antibodies of the same purity and immunoreactivity as used in these experiments (9, 11). The principle of radioimmunodetection could then be applied to other tumor-associated markers, such as AFP and hCG (12, 13), although like CEA, these are not substances which are quantitatively distinctive for cancer. Thus, for purposes of tumor detection and localization, markers which are quantitatively increased in certain neoplasms, even if also circulating in the blood, can serve as selective targets for tumor-seeking antibodies. In initial clinical trials, Ettinger et al. (5) and Order et al. (26–28) have reported success in using therapeutic doses of radioactive antibodies to ferritin or to CEA in the treatment of hepatocarcinomas.
sible for the tumor inhibition seen when very high doses of radioactive IgG are administered. Whereas as much as 2.57% of the injected radioactive antibody was present in the tumors 3 days after administration, only 0.93% of the control radioactive IgG was detected in the tumors at this time. The specific activity of the radioactive antibody (μCi/g tissue) in the tumor at this time was almost 20, whereas it was only 7.2 for the control immunoglobulin. Nevertheless, these results suggest that, with very high doses of radioactive antibodies, nonspecific antitumor effects can be achieved because of the increased accretion of IgG in tumors. On the other hand, the use of specific antibodies showing selective and significant uptake over normal IgG in tumors would afford an opportunity to achieve similar or better therapeutic effects with lower doses, particularly for micrometastases and smaller tumors which may not sequester increased amounts of normal IgG. This view is supported by our findings in 22 cancer patients receiving diagnostic doses of radioiodinated normal goat IgG. Only 4 of 32 tumor sites showed some evidence of transient radiolocalization, 3 of which were massive lesions of at least 10 cm in diameter (11).

Radiobiological calculations indicated that the tumor received, at up to 20 days after therapy, 1325 rads of 131I radiation for the specific antibody and only 411 rads for the normal IgG preparation. It is interesting to note that this relatively low tumor radiation dose is in the same range as that calculated in clinical studies by Order et al. (26–28) and which, similar to our experimental findings, showed tumor regression. However, these doses are low in comparison to the usual 6000 rads given clinically but are similar to the 1000- to 2000-rad low-dose rate "boost" therapy administered interstitially to help control bulky tumors, such as carcinoma of the cervix. It is conceivable that more tumor-restricted radiobiologicals will be useful for delivering low-dose-rate radiation to inaccessible or diffuse sites in a manner similar to current intracavitary or interstitial implantable sources for bulky disease. This would be particularly valuable for hypoxic or cell cycle-dependent radio-resistant tumors.

Because the tumor doses used in these experiments to achieve tumor regression are considerably lower than the usual therapeutic doses, it is interesting to speculate that other mechanisms may be involved in the antitumor effects achieved. For example, the attachment and lodging of CEA antibody on the tumor cell membrane may produce a highly localized dose rate and concomitant membrane damage which may be more lethal to the cell than the nuclear damage usually resulting from a more uniform dose distribution. This would provide an excellent means of producing repair-resistant damage at tumor sites while allowing more repair in the normal tissues. It is also conceivable that some synergism exists between the radiation and nonradiation effects of the specific antibody therapy, perhaps by means of the antibody making the tumor cells selectively more susceptible to the effects of ionizing radiation, or vice versa. Since the anti-CEA antibody used in these experiments did not show direct antitumor effects at a dose of up to 100 μg, other doses and application schedules need to be evaluated.

These experiments represent initial encouraging results supporting the further pursuit of radioactive antibodies to tumor-associated markers in the therapy of human cancer. More extensive investigation is now required to determine the best therapy schedule and dose rate, antibody vehicle and target, radionuclide, and related parameters which are critical to the eventual design of clinical trials. The question of combined treatment modalities, including chemo- and radioimmunotherapy, can also be investigated in this and other human tumor-animal host models having the appropriate tumor-associated markers.

Recent interest in cancer immunotherapy has concentrated on cellular immunity, where efforts have been made to enhance the host’s immune response either specifically or nonspecifically. However, the results have been for the most part disappointing. As already discussed, the role of specific passive immunotherapy is likewise controversial. It is thus indicated that the selective uptake of tumor-associated antibodies, such as against CEA, AFP, hCG, and other such cancer "markers," be exploited to selectively transport toxic agents to tumor cells, especially in patients with a low tumor burden or when hematogenic dissemination of malignant cells is prevalent. The experience to date in the administration of large doses of xenogeneic antitumor globulins to humans suggests that this is a relatively safe procedure (24, 34). Furthermore, our own work has shown that low doses of goat IgG antibodies to CEA, AFP, and hCG administered to over 350 patients for radioimmunodetection studies did not result in any toxicity or untoward reactions, even after repeated application (11). We appreciate, however, that the higher doses required for therapeutic purposes will require extensive testing of the safety of the preparations and careful monitoring of the patients being studied.

ACKNOWLEDGMENTS

We thank Michael West and Ricky Collins for excellent technical assistance.

REFERENCES

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