Ablation of Human Colon Carcinoma in Nude Mice by $^{131}I$-labeled Monoclonal Anti-Carcinoembryonic Antigen Antibody F(ab')$_2$ Fragments


*Ludwig Institute for Cancer Research, Lausanne Branch; and Institute of Biochemistry, University of Lausanne, CH-1066 Epalinges, Switzerland

Abstract

Pooled F(ab')$_2$ fragments of three MAbs against distinct epitopes of carcinoembryonic antigen (CEA) were used for radioimmunotherapy of nude mice bearing a subcutaneous human colon carcinoma xenograft. 9–10 d after transplantation when tumor nodules were in exponential growth, 36 mice were treated by intravenous injection of different amounts of $^{131}I$-labeled MAb F(ab')$_2$. All 14 mice injected with a single dose of 2,200 (n = 10) or 2,800 µCi (n = 4) showed complete tumor remission. 8 of the 10 mice treated with 2,200 µCi survived in good health for 1 yr when they were killed and shown to be tumor free. Four of nine other mice treated with four fractionated doses of 400 µCi showed no tumor relapse for more than 9 mo. In contrast, all 15 mice injected with 1,600–3,000 µCi $^{131}I$-control IgG F(ab')$_2$ showed tumor growth retardation of only 1–4 wk, and 15 of 16 mice injected with unlabeled anti-CEA MAB F(ab')$_2$ showed unmodified tumor progression as compared with untreated mice. From tissue radioactivity distributions it was calculated that by an injection of 2,200 µCi $^{131}I$-MAb F(ab')$_2$, a mean dose of 8,335 rad was selectively delivered to the tumor, while the tissue-absorbed radiation doses for the normal organs were: peripheral blood, 2,093; stomach, 1,668; kidney, 1,289; lung, 1,185; liver, 617; spleen, 501; small intestine, 427; large intestine, 367; bone, 337; and muscle, 198. These treatments were well tolerated since out of 19 mice with complete tumor remission only 4 required bone marrow transplantation and 17 were in good health for 6–12 mo of observation. The results demonstrate the selective destruction of established human colon carcinoma transplants by intravenous injection of either single or fractionated doses of $^{131}I$-MAb F(ab')$_2$.

Introduction

After the discovery of the hybridoma technology (1), which allows the production of MAbs directed against new tumor-associated antigens (2–5) and selective antigenic determinants of known tumor markers such as carcinoembryonic antigen (CEA) (6, 7), there has been renewed interest in antibody-mediated cancer therapy. Several clinical trials of cancer treatment with unlabeled antibodies involving patients with leukemia, lymphoma (8, 9), melanoma (10–13), and colon carcinoma (14) have recently been reported. Antibodies coupled to toxins were used in a therapeutic trial of melanoma patients (15). Polyclonal and monoclonal antibodies carrying a radioisotope of medium or high beta energy like $^{131}I$ or $^{90}Y$ have been used in some clinical tumor therapy trials (16–22). The theoretical advantage of such isotopes consists in their emission of electron particles that can penetrate and damage several cell layers around the targeted tumor cells. Thus, within a solid tumor nodule cells with low antigen expression can be lethally hit by electrons emitted from radiolabeled antibodies concentrated on adjacent tumor cells expressing more antigen.

We have selected for immunoscintigraphy of colorectal carcinomas several anti-CEA MAbs (23, 24) and have recently reported their use in a murine radioimmunotherapy model (25) as well as in a therapeutic approach in colorectal carcinoma patients (26). The treatment was well tolerated by the patients, but the doses of radioactivity delivered to the tumors were too low to produce tumor regression.

In nude mice grafted with colon carcinoma we have obtained specific but limited tumor regressions by using a mixture of intact MAbs and their F(ab')$_2$ fragments labeled with $^{131}I$ (25). We describe here, in the same model, definitively more successful therapeutic results obtained by the exclusive use of F(ab')$_2$ fragments of the same anti-CEA MAbs labeled with higher doses of $^{131}I$. Under stringent experimental conditions, including the initiation of therapy only 9–10 d after tumor transplantation and an intravenous mode of antibody administration, we have obtained complete remission of human colon carcinoma xenografts in a significant number of mice without altering their long-term survival. These results compare favorably with previous reports from the literature (27–39). A precise dosimetry, based on a time course study of the tissue distribution of $^{131}I$ in dissected animals, gives information that might be helpful for designing antibody-guided radioimmunotherapy trials in patients with disseminated colon carcinomas.

Methods

MAbs. F(ab')$_2$, fragments from three MAbs (MAb 35, CE-25-B7 [B7], and B17) against three independent epitopes of CEA were selected according to criteria required for therapeutic injection of patients (26, 40), including high percentage of binding to purified CEA and no reaction with (a) the cross-reacting antigens NCA-55 and NCA-95 (41, 42), (b) biliary glycoprotein (43), and (c) fresh human granulocytes. The $^{131}I$-labeled F(ab')$_2$ fragments from the three MAbs were shown to localize equally well into human colon carcinoma xenografts yielding high tumor to whole body ratios ranging from 35 to 50 (25). Preparation of F(ab')$_2$ fragments. Antibodies or IgG from ascites of the three hybridomas 35, B7, and B17, and of the IgG$_1$-secreting mouse.
myeloma P3 × 63 (1) were precipitated with ammonium sulfate, the redissolved sediment was digested with pepsin, and F(ab')2 fragments were purified as described earlier (25, 44). F(ab')2 fractions were shown to be 95–98% pure by SDS-PAGE.

Labeling of F(ab')2 with 111In. Batches of 1 mg of F(ab')2 fragments from single or pooled anti-CEA MABs were labeled by the chloramine T method with 10 μCi of 111In yielding a specific activity of 8–9 μCi/μg. Immunoreactivity of radiolabeled F(ab')2 was determined as described earlier (25).

Nude mouse tumor model and therapeutic modalities. The human colon carcinoma T380, generically provided by S. Halpern, San Diego (45), was serially transplanted subcutaneously (46) to 7–9-wk-old male "Swiss" transgenic nude mice (IFA/C3H, Carrel, L'Arbresle, France). Tumor T380 contains almost no necrotic areas, is moderately differentiated, and contains numerous pseudolumina that are rich in CEA (45).

The mice were held in aseptic conditions using filter paper-topped cages and nourished with standard, vitamin-supplemented, and irradiated food. In addition, drinking water (maintained at pH 6) was supplemented with a polyvitamin (Protovit iv, 0.1 ml/300 ml water; Roche, Basel, Switzerland) and amino acid preparations (Polievo iv, 0.05 ml/300 ml water; Pirolin AG, Horw, Switzerland). Lugol iodine (5%) solution was added into drinking water (0.2 ml/300 ml) 3 d before and up to 6 wk after injection of 131I-F(ab')2.

8–10 d after subcutaneous injection of 50 mm3 of mixed tumor T380 into the right flanks of the animals, ~90% of the transplants entered into exponential growth.

Therapy was started at day 9 or 10 after tumor transplantation. Nude mice were selected for evidence of tumor growth and randomly distributed into the different therapy and control groups. For each of the three series of experiments (described in Figs. 1–3), 111I-labeled anti-CEA MAB F(ab')2, 131I-labeled control IgG F(ab')2, and unlabeled anti-CEA MAB F(ab')2 were intravenously injected at the same day, while another control group remained untreated.

Follow-up of treated and control mice. Three different diameters of the tumors were measured twice a week for 50 d and then once a week. Tumor volume was calculated by the formula vol = πr1 × r2 × h, where r1 = radius. The precision of these measurements was found to be ±10–15% when performed by different observers, by comparison of results obtained by external tumor measurements, and by direct weighing of 20 dissected tumors.

Whole body counting was performed just after injection of 131I-F(ab')2 and every 1–2 d thereafter using an assayer (RADX Corp., Houston, TX). On the day of injection and every 2–3 d thereafter, mice were weighed until they recovered from the initial weight loss. At 4, 7, 14, 21, 28, and 35 d after injection of 131I-MAB or control F(ab')2, blood was taken from the tail vein and peripheral white blood cells (WBC) were counted.

Statistical analysis. Concerning duration of regression or cure rates, statistical analysis was performed with the t test (quantitative analysis) or with the χ2 test (qualitative analysis), respectively.

Bone marrow transplantation. Nude mice with peripheral WBC falling below 2,000 cells/mm3 were transplanted as prevention against infections with bone marrow cells (BMC) from a healthy nude mouse of the same Swiss genetic background. Donor mice were killed and BMC were washed out from the long bones (femur, tibia, and humerus) with a small volume of serum-free culture medium. 10–12 × 106 BMC were recovered from each donor mouse and 3–4 × 106 BMC were injected intravenously per recipient mouse (47).

Dosimetry for tumor and normal organs. 18 mice were injected with 10 μCi of 131I-anti-CEA MAB F(ab')2 (labeled as for treatment) together with 275 μg of unlabeled anti-CEA MAB fragments. At different times after injection, groups of three mice were killed and dissected, and the tissue distribution of radioactivity was measured in a gamma counter.

In more recent experiments eight tumor-bearing mice were injected with 400 μCi of 131I on 50 μg anti-CEA MAB F(ab')2. The mice were dissected at 4, 8, 24, and 48 h after injection, and tissue distribution of radioactivity was measured.

From the directly measured tissue radioactivity, an integral dose of μCi × h was calculated per gram of tumor and the normal organs (for the doses received after 48 h, a 24-h I/2 decrease of radioactivity was taken into account based on whole body 131I measurements). Tissue-absorbed beta radiation was calculated by the formula Dβ = 2.13 × μCi/g tissue × h × Eβ rad (where Eβ = 0.19 g/μCi for h for 131I) for tumor, normal organs, and whole body. An additional gamma radiation dose (Dγ) was assumed to be equally distributed to all normal mouse organs and tumor; Dγ, whole body dose is 10% of the Dβ whole body dose for a 30-g mouse (25, 48).

Results

Quality control of radiolabeled F(ab')2. F(ab')2 fragments of each individual and of the pool of the three selected MABs reacting with independent epitopes on the CEA molecule were radiolabeled with therapeutic doses of 131I and analyzed for their binding capacity to CEA immobilized on CNBr-activated Sepharose (Pharmacia Fine Chemicals, Uppsal, Sweden). The different labelings gave 58–75% binding after incubation with a limited excess of Sepharose-CEA, and <2% binding with human peripheral WBC.

Therapy with increasing doses of 131I-labeled MAB F(ab')2. 400, 800, 1,600, and 2,800 μCi 131I-anti-CEA MAB F(ab')2 were injected as a single dose at day 9 after tumor transplantation in four groups of four to five mice. Mice treated with 400 and 800 μCi showed tumor regression lasting for a mean of 17 d (range 12–24 d) and 24 d (range 15–33 d), respectively (Fig. 1 B). Four out of five mice treated with 1,600 μCi showed tumor regression lasting from 59 to 85 d (mean 74 d), significantly different from the groups injected with 400 or 800 μCi (2P < 0.001). In the fifth mouse a complete tumor regression persisted 6 mo after therapy. All four mice treated with 2,800 μCi showed complete tumor regression. Three of the last four mice were given bone marrow transplantations at day 7 after therapy because of WBC depression. All four mice treated with 2,800 μCi and one out of five mice treated with 1,600 μCi of 131I-F(ab')2 were tumor free and in good health 6 mo after injection, when they were killed for histologic examination.

Three control mice received an injection of 3,000 μCi of normal IgG F(ab')2 and showed minimal tumor regression that lasted for a mean time of 23 d (range 20–25 d). All three control mice required bone marrow transplantation. The tumor regressions observed after injection of 3,000 μCi normal IgG F(ab')2 were significantly shorter when compared with mice injected with 1,600 or 2,800 μCi MAb F(ab')2 with 2P < 0.001 for both comparisons.

Six mice were injected with 350 μg of unlabeled anti-CEA MAB F(ab')2 (an amount of fragments corresponding to that of the 2,800-μCi injections). Five out of six mice showed exponential tumor progression similar to that of the six untreated mice, reaching a tumor size of more than 1,000 mm3 within 20–40 d, whereas one mouse showed complete tumor regression (Fig. 1 A) (P < 0.01 comparing 2,800 μCi 131I-labeled vs. 350 μg unlabeled MAB F(ab')2).

Therapy with repeated doses of 400 μCi 131I-MAB F(ab')2. Four doses of 400 μCi 131I-MAB F(ab')2 were injected into nine mice at days 10, 12, 24, 38 after tumor transplantation. Tumor regression was observed in all nine mice, as shown in Fig. 2. In four mice, the regression is complete with no relapse.
**Figure 1.** Tumor size in mice injected with increasing single doses of $^{131}$I-MAb F(ab)${}_2$. (B) Four groups of four to five mice were injected with a single dose of 400, 800, 1,600, or 2,800 μCi $^{131}$I-anti-CEA MAb F(ab')$_2$ 9 d after transplantation of the human colon carcinoma T380. In the first two groups all tumors relapsed relatively early, while in the last group all tumors showed a complete remission over a 6-mo observation period. In the third group of five mice injected with 1,600 μCi the durations of the tumor remissions are very heterogeneous, and they are thus indicated by the five thin lines (+). (A) The thick lines indicate the evolution of the tumor size in the 15 control mice that were either untreated, treated with 3,000 μCi of $^{131}$I of control IgG F(ab')$_2$, or treated with unlabeled anti-CEA MAb F(ab')$_2$. In the last group, a single mouse showed an unexpected complete tumor remission (+). Vertical bars indicate SEM.

**Figure 2.** Tumor size in mice injected with repeated, fractionated doses of $^{131}$I-MAb F(ab)${}_2$. Three groups of mice received four injections (at times indicated by vertical arrows) of either 400 μCi $^{131}$I-anti-CEA MAb F(ab')$_2$, 400 μCi $^{131}$I-normal IgG F(ab')$_2$, or a corresponding amount (50 μg) of unlabeled anti-CEA MAb F(ab')$_2$. A fourth group of mice was untreated. The thick lines represent the mean of the tumor sizes from each group. The range of tumor sizes in control mice is shown by the dashed lines. The range of the tumor sizes in nine mice treated with the $^{131}$I-anti-CEA MAb F(ab')$_2$ is shown by two thin lines up to 60 d after transplantation; thereafter, the evolution of tumor sizes in individual mice is shown by nine thin lines.
after 9 mo of observation. These four mice were in good health with normal body weight. The five other mice showed tumor regression lasting for a mean of 60 d (range 55–70 d). Relapsing tumors in this group were mostly tumors that were bigger at the time of 131I-MAB F(ab')2 injection.

The injection of seven control mice with four repeated doses of 400 μCi 131I-normal IgG F(ab')2 had only a minimal effect on tumor growth which was retarded for 4–17 d (mean 9 d) as compared with untreated controls. Comparison of results in mice treated with 131I-anti-CEA MAB F(ab')2 and with 131I-normal IgG F(ab')2 in terms of cure rates (four of nine vs. zero of seven) as well as of regression duration in relapsing animals (60±6 d vs. 8.9±5.8 d) gave significant differences (P < 0.05 and 2P < 0.001, respectively). Four injections of the corresponding amount of unlabeled MAB F(ab')2 (50 μg) had no inhibitory effect on tumor growth in five mice.

Therapy with 2,200 μCi 131I-MAB F(ab')2 in a single dose. A group of 10 mice was treated 10 d after tumor transplantation with a single dose of 2,200 μCi 131I-anti-CEA MAB F(ab')2. All 10 mice showed complete tumor remission (Fig. 3). Eight of these mice were still tumor free and in good health 1 yr after therapy when they were killed. Two mice were killed at 4 and 8 mo after therapy because of progressive ulcerative skin disease or cachexia.

The same amount of 2,200 μCi of 131I coupled to normal IgG F(ab')2, injected in five control mice, resulted in short tumor regressions lasting for only 17–24 d (mean 21 d) (P < 0.001 when 131I-normal IgG F(ab')2 are compared with 131I-anti-CEA MAB F(ab')2). In a group of five mice injected with the corresponding amount of unlabeled anti-CEA MAB F(ab')2 (275 μg), all five mice showed unmodified tumor growth as compared with untreated mice and reached a tumor size of more than 1 g within 20–40 d after transplantation.

Calculation of radiation doses for tumor and normal tissues. 18 mice were injected with 10 μCi 131I-anti-CEA MAB F(ab')2 mixed with 275 μg of unlabeled anti-CEA MAB fragments (corresponding to the amount of fragments in the 2,200 μCi therapy group). At different times after injection (1, 4, 8, 12, 24, and 48 h), groups of three mice were killed and dissected, and the tissue distribution of radioactivity was measured in a gamma counter. Fig. 4 shows that a plateau of ~20% of injected dose per gram of tumor was obtained between 4 and 12 h after injection, with a maximum value of 20.7% at 8 h. Beyond 12 h a steady decline of tumor radioactivity with 1/2 of ~24 h was observed. Interestingly, the maximum percentage of the anti-CEA MAB F(ab')2 (20.7% = 57 μg) localized per gram of tumor (containing ~40 μg of CEA) (45) corresponds to almost a saturation of each CEA molecule of 200 kD with three F(ab')2 fragments of 100 kD.

From the tissue distributions of 131I-MAB F(ab')2 (Fig. 4) an integral dose of microcuries × hours per gram tumor and normal organs was calculated, and thereof the tissue-absorbed radiation doses (25, 48). The results indicate that for an injection of 2,200 μCi of 131I coupled to 275 μg of MAB F(ab')2, the mean radiation doses were 8,335 rad for the tumor, 2,093 rad for the blood, 1,668 rad for the stomach, 1,289 rad for the kidney, 1,185 rad for the lung, 617 rad for the liver, 501 rad for the spleen, 427 rad for small bowel, 367 rad for large bowel, and 337 rad for the bones (Table I). The radiation dose absorbed by bone marrow was not determined, but we can assume that it is similar to the 501 rad absorbed by the spleen. Such a dose of bone marrow irradiation appears consistent with the modest depression of peripheral WBC in 9 of 10 mice treated with 2,200 μCi of 131I.

In this experiment a high dose of radioactivity was delivered to the tumor with a single injection of 131I-MAB F(ab')2, but the percentage of injected dose in tumor was not optimal. After injection of lower amounts (50 μg) of the same F(ab')2 fragments a significantly higher percentage of injected dose per gram tumor was reached at 8 h (mean 36.4%±1.9%, three mice) as compared with injection of the larger amounts (275 μg) of F(ab')2 (20.7%±3.2%, three mice) (2P < 0.005) (Table I).

Figure 3. Tumor size in mice injected with a single dose of 2,200 μCi MAB F(ab')2.

Three groups of mice were injected 10 d after tumor transplantation, either with a single dose of 2,200 μCi 131I-anti-CEA MAB F(ab')2, the same amount of 131I coupled to control IgG F(ab')2, or the corresponding amount of unlabeled anti-CEA MAB F(ab')2 (275 μg). A fourth group of mice was untreated. The mean tumor size for each group is represented by thick lines. The range in tumor size in the control groups is represented by the dashed lines, while the range in tumor size of 131I-anti-CEA MAB F(ab')2-treated animals is shown by two thin lines. A mouse in the antibody-treated group killed at 4 mo because of ulcerative skin disease is indicated by t.
column 3). Therefore, possible approaches to reach optimal tumor to normal tissue ratios in therapy protocols would be to use repeated injections of $^{131}$I-Mab $F(ab')_2$ (as our results obtained in mice injected with $4 \times 400 \mu$Ci indicate) or to label Mab fragments to higher specific activities by new labeling procedures (49).

Table I. Tissue-absorbed Radiation Doses after Injection of $^{131}$I-labeled Anti--CEA MAB $F(ab')_2$ Fragments

<table>
<thead>
<tr>
<th>Organ</th>
<th>2,200 $\mu$Ci (275 $\mu$g) $F(ab')_2$ and tumor to normal tissue ratios*</th>
<th>400 $\mu$Ci (50 $\mu$g) $F(ab')_2$ and tumor to normal tissue ratios*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>8,335±</td>
<td>2,167±</td>
</tr>
<tr>
<td>Blood</td>
<td>2,093 (4.0)*</td>
<td>440 (4.9)*</td>
</tr>
<tr>
<td>Liver</td>
<td>617 (13.5)</td>
<td>137 (15.8)</td>
</tr>
<tr>
<td>Kidney</td>
<td>1,289 (6.5)</td>
<td>227 (9.5)</td>
</tr>
<tr>
<td>Lung</td>
<td>1,185 (7.0)</td>
<td>203 (10.7)</td>
</tr>
<tr>
<td>Stomach</td>
<td>1,668 (5.0)</td>
<td>330 (6.6)</td>
</tr>
<tr>
<td>Small intestine</td>
<td>427 (19.5)</td>
<td>86 (25.0)</td>
</tr>
<tr>
<td>Large intestine</td>
<td>367 (22.7)</td>
<td>70 (31.0)</td>
</tr>
<tr>
<td>Spleen</td>
<td>501 (16.6)</td>
<td>106 (20.4)</td>
</tr>
<tr>
<td>Bone</td>
<td>337 (24.7)</td>
<td>73 (29.7)</td>
</tr>
</tbody>
</table>

* The tumor to normal tissue radiation ratios are obtained by dividing the tumor radiation dose by that of each normal tissue.

† Mean calculated radiation dose in rad per organ. Data obtained from 18 tumor-bearing mice from which radioactivity tissue distribution are shown in Fig. 4.

‡ Mean calculated radiation dose in rad per organ. Data obtained from eight tumor-bearing mice injected with 400 $\mu$Ci Mab $F(ab')_2$ in a preliminary experiment.

Figure 4. Evolution of the biodistribution in tumor and normal tissues after injection of $^{131}$I-Mab $F(ab')_2$. 18 mice were each injected with $10 \mu$Ci of $^{131}$-anti-CEA MAB $F(ab')_2$ (1.2 $\mu$g protein) mixed with 275 $\mu$g of unlabeled anti-CEA MAB $F(ab')_2$. At different times (1, 4, 8, 12, 24, and 48 h) after injection, three mice were killed and the radioactivity in the tumor and normal organs was measured. The bars represent the percentages of injected dose per gram of tumor (T, black bars) and of different normal organs (shaded bars), including liver (Li), kidneys (K), lung (L), spleen (S), muscle (M), and blood (B). Vertical lines represent 1 SD. This tissue distribution analysis represents the basis for the dosimetric calculations.

Short-term toxicity after injection of radiolabeled fragments. The weight of treated and control mice was measured at days 0, 2, 4, 7, 14, and 21 after injection. The mean weight of the mice of all groups was ~ 30 g at the beginning of therapy. An average weight loss of 10% ranging from 0 to 15% was observed at day 4 after injection of 1,600 or more $\mu$Ci of $^{131}$I coupled to either antibody or control IgG $F(ab')_2$ fragments. 5% or insignificant weight loss was seen in mice injected with 800 $\mu$Ci or less of $^{131}$I-F(ab')$_2$, as well as in the mice injected with unlabeled Mab. Normal weights were recovered within 2–3 wk after injection.

Peripheral WBC were repeatedly controlled at 4, 7, 14, 21, 28, and 35 d after radioactivity injection. Untreated tumorbearing mice had 9,000–18,000 (mean 13,200, 15 mice) WBC/mm$^3$. Moderately decreased WBC values ranging from 3,500 to 9,000 (mean 5,800, eight mice/mm$^3$) were observed 2–3 wk after injection of 400–800 $\mu$Ci. After injection of 1,600 $\mu$Ci the WBC decreased to values ranging from 2,100 to 8,300/mm$^3$ (mean 3,600, five mice) already at 7 d after injection. Higher amounts of radioactivity (2,200 and 2,800 $\mu$Ci) gave more severe WBC depression with values ranging from 1,100 to 5,700/mm$^3$ (mean 3,200, 14 mice) 4–7 d after injection. Nevertheless, only 1 of 10 mice injected with 2,200 $\mu$Ci and 3 of 4 mice injected with 2,800 $\mu$Ci $^{131}$I-anti--CEA MAB fragments required bone marrow transplantation because WBC fell below 2,000/mm$^3$. More severe WBC depression with values ranging from 250 to 1,150 cells/mm$^3$ (mean 730, eight mice) was observed after injection of 2,200 and 3,000 $\mu$Ci $^{131}$I-labeled control mouse IgG $F(ab')_2$, which, as has been shown earlier (25), had a longer whole body $t_{1/2}$ than anti--CEA MAB $F(ab')_2$. Thus, all the five mice injected with 2,200 $\mu$Ci and the three mice injected with 3,000 $\mu$Ci of $^{131}$-normal IgG Ablation of Colon Carcinoma by Radioimmunotherapy 1453
F(ab\textquotesingle)\textsubscript{2} required bone marrow transplantation. Peripheral WBC values began to rise at 7–10 d after bone marrow transplantation and in transplanted as well as untransplanted mice WBC completely recovered at 3–5 wk after injection of radiolabeled MAB.

Long-term toxicity after injection of radiolabeled fragments. In all treatment groups the mice showed no toxic side effects except for the bone marrow depression. 2 of the group of 10 mice successfully treated with 2,200 μCi MAb F(ab\textquotesingle)\textsubscript{2} were killed at 4 and 8 mo after therapy because of progressive ulcerative skin disease or cachexia. The organs from several other mice killed up to 6 mo after treatment with 1,600–2,800 μCi of $^{131}$I-MAB F(ab\textquotesingle)\textsubscript{2} were examined histologically. No evidence of tissue necrosis or fibrosis was detected.

Morphology and CEA expression in relapsing tumors. Tumors from six mice relapsing at 55–75 d after therapy with 1,600 μCi $^{131}$I-MAB F(ab\textquotesingle)\textsubscript{2} administered in a single dose (experiment 1, n = 3) or in fractionated doses (experiment 2, n = 3) were analyzed histologically. In all six mice the tumor nodules showed the same differentiation degree as the original tumor. These apparently growing nodules were surrounded by areas of necrotic tumor and fibrosis. CEA expression in the numerous pseudolumina of the relapsing nodules was determined by immunocytochemistry and was as abundant as in the untreated tumors.

Histology of remaining micronodules in mice with complete remission. The small nodules remaining at the site of tumor transplantation in five successfully treated mice of experiment 1 (Fig. 1) 6 mo after therapy contained mostly fibrosis, and in three cases some rare cells of epithelial origin (encapsulated in fibrosis) with no sign of cell division. Among the 10 mice of experiment 3 (Fig. 3) who had all complete tumor remission, 2 mice were sacrificed 4 and 8 mo after therapy, and the 8 others (in good health) at 12 mo. No evidence of tumor relapse was found at autopsy. Histologic examination of the small (tumor) nodules showed fibrosis with no tumor cells in nine mice and some remaining epithelial cells (without sign of cell division) surrounded by fibrosis in the mouse that was killed at 4 mo.

Discussion

Several reports from the literature describe delayed tumor growth (27–30) and in some cases complete remission of solid tumors in nude mice by treatment with various MAbs (31–39). Complete remissions, however, were often obtained by initiating antibody treatment within 24 h after tumor transplantation, when the tumor cells are not yet established and organized (31–33, 35, 37). Thus, these tumor remissions should rather be considered as prevention of engraftment. In other experiments very large doses of radioactivity on intact antibodies were injected, which caused severe radiation toxicity and death of a high percentage of the treated animals (36, 38, 39).

In the present experiments we started therapy 9–10 d after tumor transplantation when the human colon carcinoma xenografts were well established and in exponential growth. After injection of high doses of $^{131}$I-anti-CEA MAB F(ab\textquotesingle)\textsubscript{2}, we could observe complete tumor regression and long-term survival of almost all animals. For instance, in the last group of 10 mice injected with 2,200 μCi $^{131}$I-MAb F(ab\textquotesingle)\textsubscript{2}, only 1 animal needed bone marrow transplantation, and 8 animals were observed during 12 mo in good health without tumor relapse.

The good tolerance of high doses of radioactivity appeared to be due to the exclusive use of radiolabeled F(ab\textquotesingle)\textsubscript{2} fragments. In a previous study, by using a $^{131}$I-labeled mixture of intact MAbs and F(ab\textquotesingle)\textsubscript{2} fragments of the same pool of three anti-CEA MAbs, we obtained either relatively short tumor regressions (25) or, when increasing the doses of $^{131}$I, we observed, in addition to bone marrow toxicity controlled by bone marrow transplantation, severe liver toxicity at days 55–100 after injection (unpublished data).

Surprisingly, despite the demonstration of the superiority of fragments for diagnostic immunoscintigraphy both experimentally (7, 19, 50–55) and in clinical trials (17, 19, 24), no thorough experimental radioimmunotherapy study using radiolabeled fragments has yet been reported. The only therapeutic use of radiolabeled antibody fragments was reported by Larson et al. (17), who used $^{131}$I-Fab fragments for the treatment of melanoma patients. Large repeated doses of $^{131}$I-Fab were well tolerated by the patients, but the radiation doses to the tumors were often too low, due to the rapid elimination of the low mol wt (50 kD) Fab fragment. Fab fragments of high affinity MAB appear to be the best carrier of isotopes for diagnostic immunoscintigraphy, especially when labeled with an isotope of relatively short physical $t_{1/2}$ such as $^{131}$I (24). Their too rapid elimination through the kidneys, however, limits their use for therapeutic purposes, while $^{131}$I-F(ab\textquotesingle)\textsubscript{2} fragments with their intermediate mol wt (100 kD) are shown here to be able specifically to eliminate human colon carcinoma xenografts.

In addition, in preliminary experiments five mice with larger T380 tumors measuring 350–1,500 mm\textsuperscript{2} were treated with 2,700–3,300 μCi of $^{131}$I-MAb F(ab\textquotesingle)\textsubscript{2} (adapted to the tumor volume), and the tumor sizes progressively decreased and remained stable with volumes ranging from 35 to 300 mm\textsuperscript{2}. All five mice were in good health 6 mo after therapy (results not shown).

The dosimetric calculations for the treatment reported in Fig. 3 indicate that in this nude mouse model an intravenous injection of 2,200 μCi of $^{131}$I-labeled anti-CEA MAB F(ab\textquotesingle)\textsubscript{2} could deliver 8,335 rad to the subcutaneous tumors with only 617 rad absorbed by the liver. This tumor to liver radiation ratio of 13.5 is very encouraging, and our more recent results, presented in Table I, indicate that tumor to normal tissue radiation ratios could be further increased for all organs by injections of lower amounts of F(ab\textquotesingle)\textsubscript{2} fragments.

The results obtained with 1,600 μCi injected in fractionated doses (4 × 400 μCi, four complete remissions out of nine animals) as compared with the mice injected with a single dose of 1,600 μCi (one complete remission out of five animals) indicate a slight advantage in favor of the fractionated doses. This advantage is not statistically significant ($P < 0.4$), but we interpret the results to mean that repeated injections of small doses are at least as effective as the injection of a single high dose.

The complete remission of colon carcinoma xenografts in 100% of the animals required the injection of high doses of $^{131}$I-F(ab\textquotesingle)\textsubscript{2} (2,200 μCi). The requirement of such high doses is essentially due to the very short initial whole body $t_{1/2}$ of F(ab\textquotesingle)\textsubscript{2} (~ 12 h) in the mouse and as a consequence also the relatively short $t_{1/2}$ (~ 24 h) in the tumor (Fig. 4). In patients, however, the whole body and the tumor $t_{1/2}$ of F(ab\textquotesingle)\textsubscript{2} can be definitively longer, ranging from 24 to 48 h depending on the amount of fragments injected (unpublished observation). Thus, the amount of injected $^{131}$I-F(ab\textquotesingle)\textsubscript{2} fragments required to
sterilize a tumor in the mouse cannot be directly extrapolated to the patients' situation.

In patients, however, we may encounter several additional problems. First, the bone marrow tolerance to radiation is lower in humans than in mice and the longer whole body 1/2 of F(ab')2 in patients may lead to more bone marrow toxicity. Second, the fact that CEA is present in normal tissues (such as normal gut) (56) may increase binding of antibody, and consequently radiation to these organs. Furthermore, the presence of CEA in circulation may provoke the formation of immune complexes that may accumulate in the reticulo-endothelium. In our limited clinical experience in the treatment of liver metastases from colon carcinoma by injection of large doses of 131I (100–300 mCi) linked to anti-CEA MAb (intact and F(ab')2 fragments) we have not yet obtained significant tumor remission (Delaloye et al., manuscript in preparation, and reference 26). The only limitation, however, has been bone marrow toxicity, a problem that might be overcome by using autologous bone marrow transplantation.

Without underestimating the obvious differences between clinical and experimental radioimmunotherapy, we considered it important in the present study to select experimental conditions as close as possible to the clinical situation, including a systemic route of antibody administration, the treatment of well-established human carcinoma xenografts, and the use of F(ab')2 fragments from anti-CEA MAbs which had been shown to be well tolerated in patients (26).

We have demonstrated that complete ablation of human colon carcinoma xenografts in nude mice can be obtained by a single or by repeated intravenous injections of 131I-anti-CEA MAb F(ab')2. Interestingly, the different treatments described here were well tolerated by the animals, since out of 19 mice with complete tumor remission, only 4 required bone marrow transplantation and 17 were in good health with no detectable tumor after a follow-up of 6–12 mo. The results obtained in this well-controlled experimental model may be useful as reference for the planning of radioimmunotherapy in colorectal carcinoma patients.

Acknowledgments

We thank Mrs. C. Paschoud for excellent technical assistance and Miss P. Brunet and Miss A.-F. Brunet for assistance in the preparation of the manuscript. We are grateful to Dr. J. F. Valley and Dr. S. Raimondi at the Institute of Applied Radiophysics, Ecole Polytechnique Fédérale de Lausanne, for helpful suggestions concerning radiation dosimetry, and to Mr. W. Geymeier and Mr. P. Dubied for preparation of the figures. We also want to thank Dr. M. Letarte and Dr. G. Miescher for reviewing this manuscript.

References


