Excretion of Carcinoma Products in Irradiated C3H/He Mice

Jan Vaage, Barrie Anderson, and Ann Chu

Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York 14263 [J. V.], and Departments of Obstetrics and Gynecology [B. A.] and Therapeutic Radiology [A. C.], Tufts New England Medical Center, Boston, Massachusetts 02111

ABSTRACT

Local low-dose (200 rads) γ-irradiation to both kidneys impaired the excretion of [3H]labeled tumor products and reduced the survival time of mice carrying carcinomas in the ascites form. Daily i.p. injections of cell-free ascites fluid into tumor-free mice for 3 weeks resulted in the death of 25 of 180 irradiated animals, with no deaths among 180 injected unirradiated controls. The only histologically visible effects of irradiation of the kidneys during ascites tumor growth or during i.p. injections of cell-free ascites fluid was a cloudy swelling of the tubular epithelium in the renal cortex together with excessive protein in the tubular lumens.

INTRODUCTION

Earlier studies have demonstrated that the level of specific concomitant transplantation resistance in mice became depressed as growing antigenic tumors exceeded a certain critical size (5, 7). The declining in vivo resistance was related to the accumulation of soluble tumor antigens in the blood (9). The resistance was quickly recovered once the source of excess antigen was removed (5, 7, 10) and the recovery was assisted by transfusions of immune serum (9). Some tumors, which may or may not also be immunogenic, produce nonspecific factors which stimulate neoplastic growth (11, 12, 15). Frequently, tumors produce nonspecific factors which inhibit lymphocyte reactivity (4, 14). The efficiency of the removal from the circulation and the excretion of such tumor products is therefore a factor against progression of cancer. The removal of circulating fragments and large molecules takes place mainly in the liver (6), while most of the small molecules (about M.W. 50,000 and less) are probably excreted by the kidneys. Thus, to the extent that material shed by or produced by cancerous tissue can promote malignant growth, the functional status of the organs that dispose of the materials is important.

The present study was prompted by an observation that mice treated with low-dose (50 to 200 rads) abdominal radiation against implanted ascites cancer cells died before unirradiated ascites tumor hosts. Preliminary experiments comparing the effects of thoracic and abdominal radiation during ascites tumor growth and histological examinations of visceral organs indicated that impaired renal function may have contributed to the early deaths of irradiated mice.

MATERIALS AND METHODS

Animals. The experimental animals were female C3H/He and C3Hf/He mice, 8 to 12 weeks old at the start of the experiments. The mice were raised and kept in a pathogen-free barrier colony. They carry only the following nonpathogenic enteric bacteria: Clostridium sp., Peptostreptococcus sp., Bacillus sp., and Bacteroides sp.

Tumors. The 2 MC's2 had developed spontaneously in multiparous C3H/He mice, and the OC had developed spontaneously in a multiparous C3Hf/He mouse. The 3 tumors had been converted to the ascites form by i.p. passages in syngeneic female mice. The conversions had been facilitated by the addition of 0.25 ml of cell-free ascites fluid per 10⁶ cells implanted i.p. (13).

Tumor Implantation. Ascites cells were sedimented at 150 × g, and the pellet was washed 2 times in Dulbecco's phosphate-buffered saline. Viability, determined by trypan blue exclusion, was always greater than 99%. The packed ascites cells constituted about 20% of the peritoneal fluid volume and more than 99% of the cells removed. The cells were suspended in Roswell Park Memorial Institute Tissue Culture Medium 1640 (Associated Biomedic Systems, Inc., Buffalo, N. Y.), and 10⁵ cells in 0.1 ml were injected i.p. MC solid tissue was removed from s.c. tumors in freshly killed or live anesthetized donor animals, and two 1-mm pieces of living tumor tissue were implanted s.c. in the right flank to initiate new growth. Disruption of tumor tissue to obtain suspensions of dispersed cells was accomplished with the use of 105 mesh polyester cloth (HC-1-105 screen cloth; TETKO, Inc., Elmsford, N. Y.) by means of a mechanical procedure described in detail in previous publications (7, 8). The washed suspended cells were usually about 20% trypan blue negative. The volume of s.c. tumors was calculated from the formula, V = 0.4 (ab²), where 0.4 is an empirical constant, and a and b are the larger and smaller diameters bisecting the tumor (1).

Ascites Fluid and Normal Serum. The fluid was collected from mice carrying 12- to 18 day-old implants of ascites cells. The fluid was separated from the cells by 2 centrifugations at 150 × g by 0.45-µm filtration of the supernatant. The total protein in MC1 ascites fluid was 1.74 ± 0.41 (S.D.) g/100 ml, and in OC1 ascites fluid it was 1.78 ± 0.57 g/100 ml, measured in a Beckman Model BU-2 spectrophotometer. Ascites fluid and serum were injected fresh or after 0° refrigeration for no more than 72 hr. Normal mouse serum was diluted from a normal concentration of 4.80 g total protein per 100 ml down to a concentration of 1.75 g protein per 100 ml with 0.9% NaCl solution before it was used.

Radioactive Labeling. To study the excretion of cancer cell products by the kidneys, the cells were first labeled in vivo with L-[3H]valine (New England Nuclear, Boston, Mass.). The donors of labeled cancer cells had been given daily i.p. injections of 1 µCi of L-[3H]valine from 5 days before to 8 days after the i.p. implantation of 10⁵ ascites cells. The ascites cells were removed after 10 days of growth.

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Radioactivity Assay. Samples of 10 to 50 mg of urine and 100 mg of blood were placed directly into weighed scintillation vials to which were added 2 ml of Protosol (New England Nuclear) and 0.25 ml of sterile water to reduce errors due to unequal quantities of water in urine and blood samples. Up to 25% of an aqueous solution can be added to Protosol without quenching the scintillation fluor. The vials were incubated at 55° for 3 hr, at which time were added 15 ml of scintillation fluor (160 ml of Liquifluor from New England Nuclear per gallon of Scintanalyzed toluene from Fisher Scientific Co., Fair Lawn, N. J.). The activity was counted in a Beckman Model LS-230 liquid scintillation counter.

Radiation Procedures. Animals were irradiated in a dual-opposing-source 137Cs machine (Irradiator Model 280; J. L. Shepherd, Glendale, Calif.) with a dose rate of 700 rads/min to the midplane. The collimators were 64 mm of steel with a 10-mm circular port. Scatter radiation was 4% at 5 mm from the edge of the field and 0.3% at 10 mm. Both irradiated and control mice were lightly anesthetized with the veterinary inhalation anesthetic Penthrane (Abbott Laboratories, Chicago, Ill.) in a large covered jar containing some tissue paper moistened with Penthrane and were taken out as soon as their motor movements ceased. Experimental mice were then immobilized with adhesive tape on a Lucite platform to simulate those that may exist in a patient with early advanced cancer, the experimental mice were prepared in the following manner. Each mouse received 10⁶ ascites tumor cells i.p. or 5 x 10⁵ solid tumor cells s.c. at the right shoulder. Five days after tumor implantation, one-half of the mice received a single dose of 200 rads to each kidney. The dose and the time of irradiation had been chosen after preliminary tests which compared the effects of irradiation of the thorax, the abdomen, or one or two kidneys at doses ranging from 50 to 400 rads and at times ranging from 1 week before to 1 week following tumor implantation. Irradiation 5 days after implantation gave the tumors time to enter progressive growth and was assumed to simulate a likely clinical status at the time of treatment. The dose of 200 rads was the lowest which, when given to both kidneys, would produce histopathological changes in the kidneys of tumor hosts and in the kidneys of mice given injections of cell-free ascites fluid.

The volume of ascites tumors and s.c. tumors was determined at weekly intervals. Randomly selected mice with ascites were removed from the experiments, the peritoneal contents were drained, and the volume of the packed ascites was determined (see “Materials and Methods”). The results of 5 experiments (5 mice/group) with MC1 ascites, 5 experiments (5 mice/group) with MC2 ascites, 2 experiments (10 mice/group) with MC2 solid, and 5 experiments (5 mice/group) with OC1 ascites have been combined for each tumor and are presented in Table 1. The results show that irradiation of the kidneys had little effect on the growth of the tumors but had a significant effect on the survival of ascites tumor hosts (p < 0.01). The effect of irradiation on the survival of mice with s.c. implants of MC2 was similar but not statistically significant.

To determine the effect of the soluble tumor products on survival with or without irradiation of the kidneys (200 rads), groups of 30 mice each were given daily i.p. injections of 1, 0.5, or 0.1 ml of cell-free MC1 and OC1 ascites fluid for 21 days, starting on the day after irradiation. The following irradiated and unirradiated control groups were included (30 mice each): (a) daily i.p. injections for 21 days of 1, 0.5, or 0.1 ml normal mouse serum; and (b) daily i.p. injections for 21 days of 1, 0.5, or 0.1 ml 0.9% NaCl solution.

Deaths occurred among the mice given injections of ascites fluid and irradiated as follows: (a) after 16 (1 mouse), 20 (1 mouse), and 21 (4 mice) daily injections of 1 ml MC1 ascites fluid; (b) after 15 (1 mouse), 18 (2 mice), 19 (2 mice), 20 (3 mice), and 21 (7 mice) daily injections of 1 ml OC1 ascites fluid; and (c) after 20 (1 mouse) and 21 (3 mice) daily injections of 0.5 ml OC1 ascites fluid. The survivors were observed for another 3 months without further deaths recorded. No unirradiated injected mice died, no irradiated mice receiving less than 1 ml MC1 ascites fluid or less than 0.5 ml OC1 ascites fluid per injection died, and no irradiated mice receiving normal serum or 0.9% NaCl solution died. The summarized results are presented in Table 2.

Effect of Radiation on Renal Clearance. To study the effect

### Table 1

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Treatment</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Survival time (days)</th>
<th>No. of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC1 ascites</td>
<td>200 rads</td>
<td>0.10</td>
<td>1.40</td>
<td>3.00</td>
<td></td>
<td>14 ± 3*</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>MC1 ascites</td>
<td>None</td>
<td>0.10</td>
<td>1.50</td>
<td>3.00</td>
<td></td>
<td>23 ± 3</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>MC2 ascites</td>
<td>200 rads</td>
<td>0.10</td>
<td>1.25</td>
<td>2.20</td>
<td></td>
<td>15 ± 2</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>MC2 ascites</td>
<td>None</td>
<td>0.10</td>
<td>1.30</td>
<td>2.20</td>
<td></td>
<td>25 ± 3</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>MC2 solid s.c.</td>
<td>200 rads</td>
<td>0.03</td>
<td>0.40</td>
<td>0.95</td>
<td>2.20</td>
<td>31 ± 6</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>MC2 solid s.c.</td>
<td>None</td>
<td>0.03</td>
<td>0.40</td>
<td>1.00</td>
<td>2.30</td>
<td>2.95</td>
<td>37 ± 8</td>
<td>25</td>
</tr>
<tr>
<td>OC1 ascites</td>
<td>200 rads</td>
<td>0.10</td>
<td>1.60</td>
<td>3.00</td>
<td></td>
<td>13 ± 4</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>OC1 ascites</td>
<td>None</td>
<td>0.10</td>
<td>1.60</td>
<td>3.00</td>
<td></td>
<td>21 ± 3</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± S.D.
that radiation may have on the ability of the kidneys to excrete soluble tumor products, the experimental mice were prepared in the following manner. MC2 ascites was implanted i.p. in one-half of the mice 5 days before selected groups of mice received a single dose of 200 rads to each kidney. Five days after the radiation treatment, all of the mice were given s.c. injections of 10^7 radiation-inactivated (10,000 rads) MC2 ascites cells labeled in vivo with L-[3H]valine (see "Materials and Methods"). At 30 min, at 4 hr, and again at 24 hr, urine was collected with a Pasteur pipet from the retroorbital sinus and placed in weighed vials. The data presented in Table 3 are the results of 4 separate tests. Each of the 4 groups was limited to 5 mice/test in order that urine and blood could be collected quickly before time would influence the results, particularly those obtained with the earliest, 30-min samples. Since the results of the individual tests were similar, they have been combined. The results of the 4-hr assays show that the clearance of the radioactive label from the blood was impeded in the irradiated tumor hosts compared to unirradiated tumor hosts (Group 2 versus Group 1, p < 0.05). The excretion of the label in the urine was significantly less in the irradiated tumor hosts than it was in unirradiated tumor hosts at 30 min and at 4 hr (Group 2 versus Group 1, p < 0.01). At 24 hr, the label was now higher in the urine of irradiated tumor hosts than it was in the other 3 groups, indicating delayed clearance.

The blood levels of urea nitrogen, creatinine, and uric acid in anesthetized irradiated and anesthetized unirradiated mice carrying MC2 ascites were determined in a Technicon AutoAnalyzer II. Blood samples from groups of 10 mice (1 ml from each) were taken by cardiac puncture on the day of the injection of 10^5 MC2 ascites cells and then at 1- or 2-day intervals for the next 2 weeks. Five days after tumor implantation, one-half of the remaining mice received a single dose of 200 rads to each kidney. The results, which are presented in Table 4, show that there were statistically insignificant (p > 0.05) elevations in uric acid from the first day after irradiation and in the blood urea nitrogen and creatinine from the second day after irradiation.

**Histological Examinations.** To examine the combined effects of radiation and ascites tumor growth on the histology of visceral organs, groups of 20 mice each were prepared in the following manner: (a) a single dose of 200 rads to each kidney given 5 days after the i.p. implantation of 10^5 MC2 ascites cells; (b) i.p. implantation of 10^5 MC2 ascites cells only; (c to f) radiation to 50, 100, 200, or 400 rads; (g) a single dose of 200 rads to each kidney, plus daily injection of 1 ml of cell-free ascites fluid given from 5 days before irradiation to 15 days after irradiation; and (h) injections of cell-free ascites fluid only. Each mouse was equally anesthetized with Penthrane on the fifth day of the test. Two mice from each group were killed from the second day of the test and for every second day thereafter. Histological examinations were made of the kidneys, livers, spleens, and lungs from all the mice. To prolong the life of the mice given i.p. implants of MC2 ascites cells, paracentesis was performed on the 12th day and again on the 18th day after the implantation. Accordingly, all histological specimens taken from tumor hosts after the 12th day of the test were taken after paracentesis.

Histological sections of the kidney of an unirradiated control mouse given i.p. implants of 10^5 MC2 ascites cells (Fig. 1) and of the kidney of an irradiated mouse given i.p. implants are shown in Figs. 1 and 2. The radiation was a single dose of 200 rads.
rads to each kidney given 5 days after the i.p. implantation of $10^5$ MC2 ascites cells. The kidneys were removed 12 days after tumor implantation, at which time none of the remaining mice had died of progressive tumor growth. The histological sections showed that the combined effects of tumor growth and irradiation had produced cloudy swelling of the tubular epithelium in the cortex and caused an accumulation of protein in the straight tubular lumens (Fig. 2). This effect was seen in the kidneys of some irradiated mice as early as 6 days after tumor implantation and in all irradiated mice from 7 days after tumor implantation until the time of death. Tumor growth without radiation, injections of cell-free ascites fluid without radiation, or radiation (50 to 400 rads) without tumor growth or without tumor implantation until the time of death. Tumor growth without tumor implantation and in all irradiated mice from 7 days after the kidneys of some irradiated mice as early as 6 days after epithelium in the cortex and caused an accumulation of protein and irradiation had produced cloudy swelling of the tubular sections showed that the combined effects of tumor growth after tumor implantation, at which time none of the remaining mice had died of progressive tumor growth. The histological analysis of kidney function in irradiated and unirradiated female C3H/He ascites tumor hosts

<table>
<thead>
<tr>
<th>Time after tumor implantation (days)</th>
<th>Blood urea nitrogen (mg/100 ml)</th>
<th>Creatinine (mg/100 ml)</th>
<th>Uric acid (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irradiated</td>
<td>Control</td>
<td>Irradiated</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>39 ± 6</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>37 ± 5</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>38 ± 4</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>40 ± 5</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>41 ± 6</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>44 ± 7</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>49 ± 8</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>48 ± 10</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>52 ± 7</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>51 ± 9</td>
<td>(32-46)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Numbers in parentheses, normal range. |

DISCUSSION

Previous studies have shown that antigenic tumor components or products are immunospecifically suppressive in vivo when present in excess (5, 7, 9, 10), and that tumors may also produce (a) nonspecific factors which inhibit lymphocyte reactivity in vitro (4, 14) and (b) nonspecific factors which stimulate tumor growth in vivo (11, 15) and in vitro (12). The neutralization and/or removal of such factors may therefore be functions which are of decisive importance in the host control of neoplastic growth. The present results, produced in an experimental mouse tumor model, show that even low-dose (200 rads) irradiation damaged the kidneys and delayed the excretion in the urine of labeled components of injected tumor cells (Table 3). In standard kidney function tests, blood urea nitrogen, creatinine, and uric acid were insignificantly elevated above normal range in mice that were given implants of MC1 ascites and irradiated with 200 rads to both kidneys (Table 4). Repeated injections of ascites fluid but not of normal serum would kill irradiated mice, suggesting that malignant cell products, more than just high protein and fluid load, contributed to the deaths (Table 2). This is circumstantial evidence which suggests that the retention of soluble tumor products may have been a factor in the early deaths of irradiated tumor hosts (Table 1).

Past studies of the effect of radiation on kidney function in humans and in animals have been done most often in the absence of malignant disease and mainly with doses ranging from 1,000 to 10,000 rads. Avioli et al. (2) measured renal function in 10 patients with abdominal cancer who had received cumulative treatments of 2,000 to 2,500 rads. In some patients, a decrease in renal plasma flow was observed after the first exposure to 400 rads. After the full course of treatments, a persistent (12 months) decrease in renal plasma flow and in glomerular filtration rate was seen in all patients. The authors attributed these effects to radiation and not to the cancers. Churg and Madrazo (3) studied radiation nephritis in normal adult rats exposed to doses from 1,500 to 10,000 rads. They estimated that 2,300 rads were needed to produce significant morphological changes and that the rat kidney is, in that respect, similar to the human kidney. Considering the present study in mice, it appears that the effect of radiation on the kidneys may be exacerbated by heavy functional load, e.g., due to the release of soluble tumor components or products, or under different circumstances, e.g., after extensive trauma (crush syndrome).

The irradiation of the kidneys must be assumed to have had a lethal effect on a proportion of the ascites cells in the 1-cm fields of radiation. This would have contributed an unknown quantity to the circulating soluble tumor components and to the functional load on the kidneys. The relative importance of the irradiation of the kidneys is indicated by the survival of all the unirradiated mice given injections of ascites fluid (Table 2). The soluble tumor products may in themselves be toxic and/or may cause toxicity by impeding the excretion of normal metabolic waste. An impaired excretory capacity of the kidneys may in turn exacerbate the course of neoplastic disease.

The anesthetic agent used on all of the mice in this study may also have contributed to reduce kidney function in irradiated mice by their combined nephrotoxic effects. The mice were, however, only very lightly and briefly anesthetized (the toe pinch reflex was not suppressed), and no deaths were recorded among unirradiated control mice which were both anesthetized and given injections of ascites fluid (Table 2). Although the kidneys are routinely shielded during abdominal radiation treatment, these organs may still receive low levels of irradiation, which may, at times of functional stress, be a matter of concern. Accordingly, because soluble factors produced by...
neoplastic cells may contribute to the progression of cancer, the efficient removal of such factors by the kidneys and also, to a lesser extent, by the liver, lungs, large intestine, and spleen (6), may be more important than is presently realized.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. a, mouse kidney 12 days after i.p. implantation of $10^6$ MC2 ascites cells. H & E, x 32. b, detail from a. H & E, x 320.
Fig. 2. a, mouse kidney 12 days after i.p. implantation of $10^5$ MC2 ascites cells and 7 days after irradiation with 200 rads to both kidneys. H & E, $\times$ 32. b, detail from a. Arrows, examples of protein accumulation and cellular edema (cloudy swelling). H & E, $\times$ 320.
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