Serum Therapy for Radiation-induced Impairment of Immune Resistance to Metastasis

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SUMMARY

The use of serum transfusion to remedy radiation-induced damage to established antitumor resistance was investigated in female C3H mice. The mice, which had been actively immunized against a syngeneic mammary carcinoma, were injected i.v. and s.c. with suspensions of cells from the same tumor and were then given 300 R extensive-field irradiation to the abdomen two times. Tumor cells implanted outside the irradiated area grew better in irradiated mice than in unirradiated controls. Under these experimental conditions, protection could be transferred to radiation-impaired hosts with several injections of cell-free immune serum. Transfers of normal serum provided a detectable but low degree of protection. The corrective effect of serum transfers to radiation-impaired hosts was clearly expressed against pulmonary tumor growth (i.v. challenge), provided the transfusions were started no later than the first day after the injection of the tumor cells. Serum transfusions were ineffective against the growth of tumors implanted s.c. Transfers of serum from hosts carrying large (15 mm) s.c. tumor implants had a negative effect on the resistance of irradiated recipients. The results indicate that humoral resistance factors, both normal and immune, may act against metastatic spread of solid tumors.

INTRODUCTION

Previous investigations in mice have shown that extensive-field irradiation can impair the effectiveness of established, systemic tumor-specific immunity (22). Preliminary results of attempts to ameliorate radiation-induced impairment by passive transfers of immune cells or serum were reported in recently published proceedings (19). Suurkula and Boeyd (8) have previously shown that spleen cells from immunized mice could restore radiation-damaged resistance to metastasis formation. Fidler (4) reported that the transfer of a large number (1 x 10^6) of lymphocytes from tumor-bearing mice reduced the number of metastases in X-irradiated mice, whereas a smaller number (1 x 10^7) promoted the development of metastases under similar test conditions. The experiments reported here have used preimmunized female C3H mice implanted s.c. and i.v. with a syngeneic mammary carcinoma to simulate tumor dissemination. The mice also received extensive-field radiation to the abdomen to create a condition of radiation-induced impairment of the tumor rejection response. This condition was then treated with passive transfers of lymph node cells or serum.

MATERIALS AND METHODS

Mice. All the animals used in these experiments were 8- to 10-week-old female mice of the inbred C3H/He strain, raised and kept in a pathogen-free environment.

Tumors. The mammary carcinoma had developed spontaneously in a multiparous C3H/He mouse. It had been kept in liquid nitrogen and was used in this study in the 2nd to 4th transplant generations. The fibrosarcoma had been induced in a virgin C3H/He mouse by a s.c. methylcholanthrene implant. It had been kept in liquid nitrogen and was used in this study in the 8th to 11th transplant generations. Both tumors had previously been shown to induce transplantation resistance in syngeneic mice (14).

Tumor Implantation. Tumor tissue was removed from freshly killed or live anesthetized donor animals, and skin and necrotic tissue were removed before the tissue was placed in culture medium in a sterile Petri dish kept on a bed of crushed ice. Implantation of two 1-cm pieces of living tumor tissue s.c. was used to initiate antitumor immunity in donors of immune lymph node cells and immune serum. Removal of sensitizing tumors implants s.c. was done under pentobarbital anesthesia. A circular incision was made in the skin around the edge of the tumor, and the tumor was removed by blunt dissection. The incision was closed with wound clips.

In all of the experiments reported here, s.c. sensitization procedures were done on the right side of the experimental animals, and s.c. challenge implantations were done on the left side. Challenges (s.c. and i.v.) were made with suspensions of tumor cells in Roswell Park Memorial Institute Culture Medium 1640. Disruption of tumor tissue to obtain suspensions of dispersed, single cells was accomplished with the use of 105 mesh polyester cloth (HC 7-105; TETKO, Inc., Elmsford, N. Y.) by a mechanical procedure described in previous publications (14, 16). The proportion of viable (trypan blue negative) cells in the tumor cell suspensions was usually about 25% or less. In each separate experiment the challenge of all the mice was completed within the briefest possible time, with the use of cells from the same
Lymph Node Cells. The inguinal, brachial, and axillary lymph nodes were removed from mice from which bilateral s.c. sensitizing tumor implants had been surgically removed 4 days previously. The sensitizing tumors were about 8 mm at the time of removal.

The lymph nodes were minced with scissors in cold culture medium, and the cells were dislodged by gently drawing the pieces into a 1-ml syringe 20 to 30 times. The suspended fragments that could pass through a 20-gauge needle were withdrawn and washed once in cold culture medium. The cell suspensions were kept on crushed ice during counting and diluting procedures and were then brought to 37°C and injected i.p.

Serum. Blood was collected by right cardiac puncture, and the serum was separated after 2 hr at room temperature. The serum was kept in the refrigerator on crushed ice.

Lymph Node Cells or Serum. To study the effects that radiation may have on host antitumor resistance in addition to therapeutic effects, and to study the effects under conditions simulating those that may exist in a patient with an antigenic neoplastic growth, we prepared the experimental mice in the following manner. The mice had been presensitized to simulate the patient with the antigenic tumor and then received living tumor cells i.v. to simulate dissemination to the lungs. Next, radiation was given to the abdomen to determine the secondary effect of radiation on the growth of antigenic tumor cells located outside an irradiated area.3 In 3 separate but similar experiments, all the mice were given the 1st sensitizing s.c. injection of 5 mg (about 5 x 106 cells) of radiation-inactivated (5000 R) tumor cells in 0.9% NaCl solution 14 days before challenge and the 2nd s.c. injection 7 days before challenge. The level of resistance of each mouse was tested with challenge implants of suspended living mammary carcinoma cells. (The day of challenge has been designated Day 0 in all experiments.) Each mouse received a total of 1 x 106 (about 20% viable) cells i.v. via the tail vein. (The presensitizing and i.v. challenge procedures were the same in each experiment of this investigation.) In 1 of the 3 experiments the mice received 400 R of radiation, and in 2 experiments they received 300 R, of the abdominal region, on each of 2 consecutive days. The results of the 3 experiments have been combined and are presented in Table 1. The mice were irradiated on Day 0 and on Day 1. Lymph node cells were transferred to irradiated recipients after the last exposure (Group 2) or before each exposure (Group 3). The experiments were terminated from 18 to 21 days after challenge.* Frequent examinations for signs of dyspnea decided the termination time. The mice were killed by CO2 asphyxiation, and the incidence and the amount of tumor growth were determined. To describe the amount of tumor growing in the lungs, where accurate measurements were not practical, values from 0 to 3 were assigned in blind readings, according to the number and size of growths found at autopsy.

Under these experimental conditions, transfers of immune lymph node cells after radiation exposure or transfers of serum could at least partially restore radiation-impaired immune resistance. Passive transfers of 0.5 ml of antitumor serum on 4 consecutive days, although not as effective as 2 transfers of 5 x 106 lymph node cells after the recipients had been irradiated, were still able to reduce tumor growth in the lungs from an incidence of 70 to 45%. Transfers of normal serum reduced the tumor incidence from 70% to 55%. Although the difference between 70 and 55% was not statistically significant, the results of the following experiments showed that the effect of normal serum treatment was consistent.

RESULTS

Repair of Radiation-impaired Immunity with Transfers of Lymph Node Cells or Serum. To study the effects that radiation may have on host antitumor resistance in addition to therapeutic effects, and to study the effects under conditions simulating those that may exist in a patient with an antigenic neoplastic growth, we prepared the experimental mice in the following manner. The mice had been presensitized to simulate the patient with the antigenic tumor and then received living tumor cells i.v. to simulate dissemination to the lungs. Next, radiation was given to the abdomen to determine the secondary effect of radiation on the growth of antigenic tumor cells located outside an irradiated area.3 In 3 separate but similar experiments, all the mice were given the 1st sensitizing s.c. injection of 5 mg (about 5 x 106 cells) of radiation-inactivated (5000 R) tumor cells in 0.9% NaCl solution 14 days before challenge and the 2nd s.c. injection 7 days before challenge. The level of resistance of each mouse was tested with challenge implants of suspended living mammary carcinoma cells. (The day of challenge has been designated Day 0 in all experiments.) Each mouse received a total of 1 x 106 (about 20% viable) cells i.v. via the tail vein. (The presensitizing and i.v. challenge procedures were the same in each experiment of this investigation.) In 1 of the 3 experiments the mice received 400 R of radiation, and in 2 experiments they received 300 R, of the abdominal region, on each of 2 consecutive days. The results of the 3 experiments have been combined and are presented in Table 1. The mice were irradiated on Day 0 and on Day 1. Lymph node cells were transferred to irradiated recipients after the last exposure (Group 2) or before each exposure (Group 3). The experiments were terminated from 18 to 21 days after challenge.* Frequent examinations for signs of dyspnea decided the termination time. The mice were killed by CO2 asphyxiation, and the incidence and the amount of tumor growth were determined. To describe the amount of tumor growing in the lungs, where accurate measurements were not practical, values from 0 to 3 were assigned in blind readings, according to the number and size of growths found at autopsy.

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Serum Treatment of Radiation-impaired Resistance to Pulmonary and s.c. Tumor Growth. The next several experiments were designed to determine whether treatment with serum after abdominal irradiation would ameliorate radiation-impaired resistance equally in different implantation sites (lungs and s.c.), to determine the effectiveness of serum treatment at different times after irradiation, and to compare the effects of serum from differently prepared donors.

Table 2 presents the combined data from 2 separate but similar experiments. Irradiation was given in doses of 300 R each, on Days 0 and 1. The presensitizing and i.v. challenge procedures were the same as for the preceding experiment and all following experiments. In addition, the mice were given 5 x 106 (about 20% viable) tumor cells s.c. at the left shoulder to compare, in 2 different anatomical sites, the effects of radiation damage and remedies. The s.c. challenge was also included in all of the following experiments.

The results presented in Table 2 show that treatment with immune serum was able to repair, to a significant degree,
The effects of abdominal region irradiation and treatment with lymph node cells or serum on mammary carcinoma growth in lungs

<table>
<thead>
<tr>
<th>Group</th>
<th>Radiation on days</th>
<th>Treatment</th>
<th>Tumor incidence/total no. of mice</th>
<th>Av. growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>None</td>
<td>14/20 (70)e</td>
<td>1.10</td>
</tr>
<tr>
<td>2</td>
<td>0.1</td>
<td>Lymph node cells, Days 1 and 2</td>
<td>6/15 (40)</td>
<td>0.73</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>Lymph node cells, Days 0 and 1</td>
<td>10/15 (67)</td>
<td>1.13</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>Immune serum, Days 0-3</td>
<td>9/20 (45)</td>
<td>0.90</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>Normal serum, Days 0-3</td>
<td>11/20 (55)</td>
<td>0.95</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>None</td>
<td>11/30 (37)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* The mice received 5 × 10⁴ lymph node cells or 0.5 ml serum per inoculum. Radiation of the abdominal area was 300 or 400 R per exposure. The mice had been presensitized with 2 s.c. injections of 5 mg of radiation-inactivated tumor cells, 14 and 7 days before challenge.

** The mice were challenged on Day 0 with 1 × 10⁶ (20% viable) tumor cells injected into the tail vein. The differences in average tumor growth in the lungs were evaluated by Student’s t test. Group 6 differs significantly only from Groups 1 and 3; there is no significant difference among Groups 1 to 5.

* Numbers in parentheses, percentage.

Table 2

The effects of abdominal region irradiation and serum treatment on mammary carcinoma growth in lungs and s.c.

** The mice received 0.5 ml serum per inoculum. Radiation of the abdominal area was 300 R per exposure, on Days 0 and 1.

** The mice were challenged on Day 0 with 1 × 10⁶ (20% viable) tumor cells injected into the tail vein and 5 × 10⁶ (20% viable) tumor cells injected s.c. at the left shoulder. The differences in average tumor growth were evaluated by Student’s t test. For the i.v. challenge, Groups 1 and 2 differ significantly from each of Groups 3, 5, and 6; Group 3 differs significantly from Groups 1, 2 and 4; and Group 4 differs significantly from Groups 3 and 6. For the s.c. challenge, Group 3 differs significantly only from Groups 1 and 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of mice with tumor/total no. of mice</th>
<th>%</th>
<th>Av. growth</th>
<th>No. of mice with tumor/total no. of mice</th>
<th>%</th>
<th>Av. size (mm)</th>
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<tbody>
<tr>
<td>1</td>
<td>Untreated</td>
<td>20/20</td>
<td>100</td>
<td>1.40</td>
<td>19/20</td>
<td>95</td>
<td>3.50</td>
</tr>
<tr>
<td>2</td>
<td>Irradiated</td>
<td>20/20</td>
<td>100</td>
<td>1.70</td>
<td>20/20</td>
<td>100</td>
<td>3.90</td>
</tr>
<tr>
<td>3</td>
<td>Sensitized</td>
<td>7/20</td>
<td>35</td>
<td>0.40</td>
<td>11/20</td>
<td>55</td>
<td>2.00</td>
</tr>
<tr>
<td>4</td>
<td>Sensitized, irradiated</td>
<td>13/20</td>
<td>85</td>
<td>1.05</td>
<td>14/20</td>
<td>70</td>
<td>2.60</td>
</tr>
<tr>
<td>5</td>
<td>Sensitized, irradiated, normal serum, Days 0-3</td>
<td>8/20</td>
<td>40</td>
<td>0.75</td>
<td>14/20</td>
<td>70</td>
<td>2.65</td>
</tr>
<tr>
<td>6</td>
<td>Sensitized, irradiated, immune serum, Days 0-3</td>
<td>6/20</td>
<td>30</td>
<td>0.60</td>
<td>16/20</td>
<td>80</td>
<td>3.10</td>
</tr>
</tbody>
</table>

* The mice received 5 × 10⁴ lymph node cells or 0.5 ml serum per inoculum. Radiation of the abdominal area was 300 or 400 R per exposure. The mice had been presensitized with 2 s.c. injections of 5 mg of radiation-inactivated tumor cells, 14 and 7 days before challenge.

** The mice were challenged on Day 0 with 1 × 10⁶ (20% viable) tumor cells injected into the tail vein and 5 × 10⁶ (20% viable) tumor cells injected s.c. at the left shoulder. The differences in average tumor growth were evaluated by Student’s t test. Group 6 differs significantly only from Groups 1 and 3; there is no significant difference among Groups 1 to 5.

* Numbers in parentheses, percentage.

Table 3 presents the combined data of 3 separate but similar experiments, with the exception that Group 5 was included in only 2 of the experiments. The experiments were essentially the same as those represented by Table 2, with the additional use of sera taken from mice carrying large (15 mm) s.c. implants of the mammary carcinoma and from mice sensitized against an antigenically unrelated fibrosarcoma. The results presented in Table 3 repeat the observations presented in Tables 1 and 2 and, in addition, show that the unrelated antitumor serum was no more effective than normal serum in reducing the growth of mammary carcinoma cells in the lungs. The results also show that all immune antitumor activity as well as the activity apparently present in normal serum was absent or inactive in the serum of mice bearing large s.c. mammary carcinoma implants. Again, the serum treatment did not noticeably benefit the recipients against tumor cells implanted s.c.

The next experiment was designed to determine the time, relative to the challenge, at which immune serum should be transferred to irradiated recipients in order to provide the best protection against implanted tumor cells. Table 4 presents the results of 4 separate but similar experiments. The values for Groups 1 to 5 are the results of 3 experiments; those for Groups 1, 2, and 5 contain the results of an additional 4th experiment. The mice were presensitized, challenged, and irradiated as in the previously described experiments. The mice were then given 0.5-m1 aliquots of
immune serum i.p., on 3 consecutive days as indicated in Table 4.

The results show that tumor growth in the lungs was not significantly inhibited unless the passive transfer was started before the 2nd day after challenge, and it was ineffective when serum treatment was delayed until the 3rd day after challenge. As in the previously described experiments, the serum treatment did not have a noticeable effect on the growth of tumor cells implanted s.c.

DISCUSSION

Previous work has shown that humoral immune factors may be primarily or particularly effective against tumor cells disseminating via the vascular system (15, 18) and that impairment of immune protection by iatrogenic factors such as irradiation (22) could promote the establishment and growth of disseminated tumor cells. In the experiments reported here, where the effects of immune cells and immune serum were compared in passive transfers of immune resistance to radiation-impaired mice, immune cells were invariably more effective (Table 1, Group 2 versus Group 4). However, although cellular transfer of tumor immunity is a well-established phenomenon, the effects of serum transfers have been less extensively investigated. For this reason, the investigation reported here has mainly considered the role of humoral factors in host resistance to cancer.

The effect of extensive-field abdominal irradiation on existing host resistance was sufficient to facilitate the establishment of tumor cells in the lungs after i.v. injection. Damage to lymph node cells must have been at least part of the reason for impairment of host resistance by irradiation.

Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment*</th>
<th>i.v. challengea</th>
<th>s.c. challengeb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of mice with tumor/total no. of mice</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Sensitized, irradiated</td>
<td>23/30</td>
<td>77</td>
</tr>
<tr>
<td>2</td>
<td>Sensitized, irradiated, normal serum, Days 0–3</td>
<td>19/30</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>Sensitized, irradiated, anti-FS serum, Days 0–3</td>
<td>17/30</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>Sensitized, irradiated, anti-MC serum, Days 0–3</td>
<td>12/30</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>Sensitized, irradiated, MC host serum, Days 0–3</td>
<td>19/20</td>
<td>95</td>
</tr>
</tbody>
</table>

* See Table 2, Footnote a. FS, fibrosarcoma; MC, mammary carcinoma.

Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment*</th>
<th>i.v. challengea</th>
<th>s.c. challengeb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of mice with tumor/total no. of mice</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Sensitized, irradiated</td>
<td>17/20</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>Sensitized, irradiated, serum Days 0–2</td>
<td>11/20</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>Sensitized, irradiated, serum Days 1–3</td>
<td>8/15</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>Sensitized, irradiated, serum Days 2–4</td>
<td>9/15</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>Sensitized, irradiated, serum Days 3–5</td>
<td>16/20</td>
<td>80</td>
</tr>
</tbody>
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* See Table 2, Footnote a.

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resistance could be at least partially restored with injections of serum, it seems that host resistance depended at least in part on molecular serum factors. Since the level of roentgens needed to inactivate isolated proteins such as enzymes is $10^8$ to $10^9$ (6), the reduced antitumor functions in mice following exposure to 600 to 800 R was probably not caused by a direct effect of radiation on molecular factors. It seems more probable that the effect was the direct result of radiation-impaired synthesis of serum factors, which have a rapid turnover, and/or was secondary to the release of destructive or interfering substances from radiation-damaged cells.

Since transfers of both normal and immune serum were shown to benefit host resistance, it appears that there may be present in serum both specific and nonspecific products that can act against disseminating cancer cells. These factors probably act quickly on cancer cells released into circulation, because protection by serum transfers was not discernible when the serum was given later than the 1st day after i.v. injection of tumor cells (Table 4). This agrees with previously reported studies on the critical importance of time in immune protection against the development of metastases (21).

The lack of activity in serum from mice carrying large tumors (Table 3, Group 5) was not surprising, since the specific abrogation in vitro of lymphocyte functions (1-3, 5, 7) and antibody functions (9-11) by serum containing soluble tumor factors, and the specific abrogation of resistance factors in vivo by excess circulating antigen during progressive neoplastic growth (12-14, 17, 19, 20, 23) have been well and repeatedly documented by several investigators.

It is difficult to explain why the transfers of serum were more effective in protecting the recipient against the implantation or growth of tumor cells after i.v. injection than against tumor cells implanted s.c. It may simply have been that immune resistance factors were more readily and more abundantly available against tumor cells injected into the blood.

The experiments discussed above may have relevance to clinical circumstances where a neoplasm is likely to have disseminated and where transfers of normal or immune serum could conceivably alleviate iatrogenic or cancer-related impairment of existing host resistance.

REFERENCES

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