Concomitant Immunity and Specific Depression of Immunity by Residual or Reinjected Syngeneic Tumor Tissue

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SUMMARY

Mice given injections of living methylcholanthrene-induced fibrosarcoma cells after the complete surgical removal of a growing, sensitizing tumor implant showed a strong immune resistance to the growth of the injected cells. If the sensitizing tumor was left in situ until 4 days after the challenge injection of tumor cells or if the tumor was killed with irradiation and left to regress, the immune resistance of the host was impaired but not abrogated. The depression was specific, since the presence of a growing, antigenically unrelated tumor did not impair the resistance to the sensitizing fibrosarcoma. Injection of graded doses of killed tumor cells of the fibrosarcoma and of the antigenically unrelated tumor, a mammary carcinoma, showed that, at moderate and low doses, only the injection of fibrosarcoma cells depressed host resistance. Large doses of either tumor depressed host resistance, probably in part because of nonspecific toxic depression.

INTRODUCTION

In the preimmunogenetics period of experimental tumor immunology, before the development of genetically uniform strains of mice made it possible to distinguish tumor-specific transplantation antigens from normal histocompatibility antigens, Ehrlich (7) described, in 1906, a phenomenon whereby mice carrying a progressively growing tumor implant would reject a 2nd implant of the same tumor. In 1908, Bashford (3) described similar findings and gave the phenomenon the name "concomitant immunity." After that, the study of antitumor resistance in tumor-carrying hosts ceased, while other approaches persisted. In 1964, Riggins and Pilch (24) found strong immune resistance to a methylcholanthrene-induced fibrosarcoma when the murine methylcholanthrene-induced fibrosarcoma at the time of inoculation. These investigators also reported that immune lymphocytes were not detectable in the spleens of athymic chontrous tumors until 3 weeks after the removal of the primary tumor. Kaibara et al. (14) reported that resistance against the MH-134 hepatoma was not detectable in mice with s.c. implants of the same tumor but that resistance developed shortly after resection of the sensitizing tumor.

Using the colony inhibition test (11) for cellular immunity, Barski and Yoon (2) found that the growth-inhibiting activity disappeared from the peritoneal exudate cells of mice carrying large s.c. implants of a Rauscher virus-infected subline of T5 tumor cells. The immunological activity of the peritoneal exudate cells reappeared about a week after surgical removal of the tumors.

The preceding reports concluded that host resistance was absent because of immunological exhaustion or specific tolerance during the persistence of the primary sensitizing tumor. In the light of other reported evidence, however, it seems likely that once immune resistance is evoked the resistance factors are never absent but may be depressed and not revealed, depending upon the conditions of the tests and the strength of the antigens.

Old et al. (20) showed that the ability of lymphoid cells to adoptively transfer immunity was reduced and was slow to manifest itself in the recipients when the donor mice were carrying progressively growing tumors. Much smaller numbers of lymphocytes from immunized mice that did not carry tumors were effective immediately. Deichman and Kluchereva (5) found strong and specific resistance to SV40 tumor challenge in tumor-carrying hamsters 5 to 10 days after their own SV40-induced tumors had become palpable. Gershon et al. (9) reported that concomitant immunity was observed in hamsters carrying 3-week-old implants of an allotransplantable lymphoma, and that spleen cells from the same animals would adoptively transfer immunity to normal recipients. Further tests reported by Gershon et al. (10) showed that removal of the sensitizing tumor implant as early as the 7th day of the course of immunization gave a low degree of immunity and increased incidence of metastatic tumor growth, whereas animals carrying the sensitizing tumor for a longer period of time developed a high degree of immunity and no metastases.

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In a study of host resistance to autografts of methylcholanthrene-induced sarcomas in rats, Takeda et al. (31) found resistance to challenge implants of antigenic tumors in autochthonous as well as in syngeneic tumor-carrying hosts. The resistance was more strongly expressed if the primary tumor had been killed by ligation at the time of the challenge implant than if the primary tumor was still intact. Using the colony inhibition technique, Hellström et al. (11) found that the lymph node cells from mice bearing progressively growing methylcholanthrene-induced sarcomas strongly reduced the ability of autochthonous target tumor cells to grow in culture. Lausch and Rapp (15) reported that hamsters bearing progressively growing, sensitizing allografts of a dimethylbenzanthracene-induced sarcoma were able to reject challenge implants of the same tumor 7 and 16 days after receiving the sensitizing implants. Vaage and Weiss (38) found that mice responded to tumor-specific immunotherapy while carrying autochthonous mammary carcinomas in situ. Vaage (34) and Vaage et al. (37) found strong resistance against syngeneic mouse mammary carcinomas in hosts which were challenged with suspensions of living tumor cells at the time of removal of immunizing tumor grafts.

In an investigation with human cancer patients, Southam (28) reported that a terminal cancer patient with squamous carcinoma of the breast who received an allograft of cultured human tumor cells developed immunity sufficient to reject subsequent grafts of the same tumor, while the primary sensitizing implant persisted until the patient died. Most of the foregoing investigative results indicate that, even in hosts with progressively growing tumors, immune resistance factors are active and although the resistance factors may be impaired by the antigenic excess of the primary tumor mass, they may still suffice to prevent the growth of injected suspensions of tumor cells or naturally disseminated tumor cells.

The present study was instigated by an observation that mice cured of a syngeneic tumor implant by excision were more resistant to challenge implants of the same tumor than were mice cured by local radiotherapy (36). This study has attempted to provide information on the antigenic specificity of the impairment of host resistance by residual or injected tumor tissue and on the relationship between degree of depression and antigenic dose.

**MATERIALS AND METHODS**

**Mice.** All the animals used in these experiments were 12-week-old female mice of inbred strain C3Hf/Bu from the defined-flora,3 pathogen-free breeding colony maintained by the Section of Experimental Radiotherapy of the University of Texas M. D. Anderson Hospital and Tumor Institute at Houston.

**Tumors.** The fibrosarcoma had been induced in a female C3H mouse by methylcholanthrene (30). It had been kept in liquid nitrogen and was reintroduced into syngeneic mice to be used in these experiments in the 3rd and 4th transplant generations for sensitizing implantations and for preparation of tumor cell suspensions.

The mammary carcinoma had developed spontaneously in a multiparous C3H/He mouse (26). It had also been kept in liquid nitrogen and was reintroduced into syngeneic mice to be used in the 3rd and 4th transplant generations.

**Tumor Implantation.** The s.c. implantation of 1- x 1-mm, pieces of living tumor tissue, was used to initiate tumor growth for the purpose of immunization. An incision was made in the skin of the right flank, and a tumor piece was placed under the skin by means of a trocar. The incision was closed with a wound clip.

Challenge implantation, in the left sides of presensitized and control mice, was by s.c. injection of viable (trypan blue-negative) tumor cells suspended in TC Medium 199 (Difco Laboratories, Inc., Detroit, Mich.), consisting predominantly of single cells and a few clumps of up to 10 cells each. The mechanical preparation of single-cell suspensions from tumor tissue has been described elsewhere (35).

**Surgical Procedures.** Removal of tumors implanted s.c. was done under Nembutal anesthesia. A circular incision was made in the skin around the edge of the tumor, and the tumor was removed by blunt dissection. Large blood vessels were cauterized. The incision was closed with wound clips.

**Radiation Procedures.** The mice with sensitizing tumor implants killed by irradiation were treated under Nembutal anesthesia on a parallel, opposing, dual-source 137Cs machine with a field size 3 cm in diameter. The dose rate was 1050 rads/min, and the total dose to the tumor was 8000 rads in a single treatment. A circular brass clamp 3 cm in diameter excluded the body of the tumor host from the field of exposure during irradiation. Scatter radiation was 3% at 0.5 cm from the edge of the field and 0.3% at 1 cm. The scatter radiation had no detectable effect on the immune status of the mice.

Tumor cell suspensions were killed by exposure to 10,000 rads on the 137Cs machine. The cell suspensions were then packed by low-speed centrifugation and resuspended in 0.9% NaCl solution in the appropriate dilutions to give the graded wet weight amounts injected.

**Statistical Analyses.** The effect of treatment is described in terms of differences in tumor incidence and in tumor size following challenge.

For comparison of tumor incidence, the χ2 test was used to evaluate the results of the test for antigenic cross-reactivity between a fibrosarcoma and a mammary carcinoma (Table 4); the test for linear trend in proportions (27) was used for all other evaluations.

Differences between groups were considered significant only when the p value of comparison was 0.05 or less.

**RESULTS**

**Effect of Residual Tumor Tissue on Host Resistance.** Female C3Hf/Bu mice received s.c. sensitizing implants of living tumor tissue in the right flank about 3 weeks before receiving the challenge injections of tumor cells. The mice with

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3 The mice carry only the following enteric bacteria: *Clostridium* sp., *Bacteroides* sp., *Peptostreptococcus* sp., and *Bacillus* sp.
sensitizing tumors to be removed or killed by irradiation 4 days before challenge received their implants 8 days before the mice that were treated 4 days after challenge. This was done in order that the tumors would be of nearly equal size at the time of treatment. To ensure that all of the sensitized mice had been exposed to the tumor antigens for an equal length of time at the time of challenge, all except the untreated control mice received 1 s.c. injection of a suspension containing 5 mg, wet weight, of irradiation killed tumor cells when the first sensitizing tumors were implanted. [The injection of 5 mg of killed tumor cells will initiate a primary immune response which is detectable in tests of resistance to tumor challenge only after a secondary stimulation (J. Vaage, unpublished observation).] This procedure is shown in Chart 1 and was used throughout the course of this investigation.

About 3 weeks after implantation, when they had reached an average size of about 10 x 10 mm, the sensitizing tumors were either surgically resected or were given a local dose of lethal irradiation and left to regress. The process of regression or necrosis and sloughing of the irradiated tumors was completed in about 2 weeks. Group 2 was included to determine whether the scatter radiation received by the host during the local irradiation of the sensitizing tumor had any effect on its immune resistance. Immediately following surgical removal of the tumor, the mice of Group 2 received 8000 rads of local irradiation to the skin adjacent to the closed incision.

The level of resistance of the variously treated mice was then tested with challenge implants consisting of $10^5$ suspended living tumor cells injected s.c. at the left shoulder and at the left hip. The groups of unsensitized control mice were challenged with cell suspensions containing from $3.3 \times 10^5$ to $3.3 \times 10^6$ living tumor cells per inoculum to determine whether the challenge dose of $10^5$ cells was within the limits of an excessive and an insufficient dose. The incidence of tumors at the injection sites was then checked at weekly intervals, and their size was measured with calipers and recorded from the time they became palpable.

Each test was terminated when some of the mice in any group became cachectic because of progressive tumor growth. The average values per group at the last recording of tumor sizes are presented in the data.

Table 1 shows that presensitized mice were particularly resistant to challenge if the sensitizing fibrosarcoma implants had been surgically removed 4 days before challenge. The groups of mice with tumors irradiated 4 days before challenge or 4 days after challenge and the mice that had tumors surgically removed 4 days after challenge all were more resistant than the untreated control mice but were less resistant than the mice in Group 1. These differences are expressed in the number of mice that developed tumors following challenge, in the total numbers of tumors, and in the average tumor size. The scatter radiation received by the mice during local radiotherapy did not affect the immune resistance.

Statistical evaluation showed that the differences in total number of tumors between untreated control mice, mice with residual tumor tissue, and surgically cured mice were significant. Because of the variability in the growth rate of individual tumors, the differences in average tumor size between groups were mainly not significant by standard statistical tests, in this and other experiments. The persistent trend of the differences in average tumor size between

### Table 1

**The effect of a growing or irradiation-killed fibrosarcoma on the resistance of the tumor host**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Challenge&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cells injected</td>
<td>No. of mice with tumors/ no. challenged</td>
</tr>
<tr>
<td>1</td>
<td>Surgery at −4 days</td>
<td>$1 \times 10^5$</td>
</tr>
<tr>
<td>2</td>
<td>Surgery and irradiation at −4 days</td>
<td>$1 \times 10^5$</td>
</tr>
<tr>
<td>3</td>
<td>Irradiation at −4 days</td>
<td>$1 \times 10^5$</td>
</tr>
<tr>
<td>4</td>
<td>Surgery at +4 days</td>
<td>$1 \times 10^5$</td>
</tr>
<tr>
<td>5</td>
<td>Irradiation at +4 days</td>
<td>$1 \times 10^5$</td>
</tr>
<tr>
<td>6</td>
<td>Unsensitized</td>
<td>$3.3 \times 10^5$</td>
</tr>
<tr>
<td>7</td>
<td>Unsensitized</td>
<td>$1 \times 10^5$</td>
</tr>
<tr>
<td>8</td>
<td>Unsensitized</td>
<td>$3.3 \times 10^5$</td>
</tr>
</tbody>
</table>

<sup>a</sup> The sensitizing tumors were implanted s.c. in the right flank 22 days before treatment. Treatment consisted of (a) surgical removal of the tumors, (b) surgical removal of the tumors plus irradiation (see text), or (c) lethal irradiation of the tumors, 4 days before (−4 days) or 4 days after (+4 days) challenge implantation of a suspension of cells of the same tumor. Average tumor size at the time of treatment was 12 x 12 mm.

<sup>b</sup> All mice were challenged with s.c. injections of tumor cells at the left shoulder and hip. Groups 1 + 2 versus Groups 3 to 5 versus Group 7, $p < 0.001$. 

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Concomitant Immunity

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variously treated and untreated groups indicates however, that this parameter is also a valid indicator of host resistance.

Table 2 presents the results of a similar test with the mammary carcinoma. The differences in number of tumors between groups are not as great in this test as in the test with the fibrosarcoma, but the effect which is expressed is statistically significant. Early and complete tumor removal results in fewer and smaller tumors developing from the challenge implants.

The Specificity of Depression of Resistance by Residual or Injected Tumor Tissue. This experiment was designed to test whether the presence of a tumor mass, antigenically unrelated to the mammary carcinoma, could depress the resistance against another tumor, sensitizing, tumor.

To test the mammary carcinoma and the fibrosarcoma for antigenic relationship, we immunized the mice against one or the other of the 2 tumors by s.c. implants in the right flank. After the tumor implants had grown to about 12 x 12 mm, they were surgically removed. Four days after these implants were removed, each of the 2 sensitized groups of mice were divided in half. Into the left flank of one half, 10⁶ living cells prepared from 1 of the implanted tumors were then injected s.c., and 10⁵ cells prepared from the other tumor were injected into the other half. Both tumors were tested for their cross-reactivity vis-à-vis each other at the same time. Groups of untreated control mice were also given injections at the same time with the same tumor cell suspensions. The incidence of tumors at the injection sites was then checked at weekly intervals.

Table 3 shows that the mammary carcinoma induced a specific resistance which protected against challenge with the mammary carcinoma but not against challenge with the fibrosarcoma. Conversely, the fibrosarcoma induced resistance against itself but not against the mammary carcinoma. By this test for antigen sharing between tumors, it was demonstrated that these 2 tumors are antigenically distinct and, therefore, that one tumor would not be expected specifically to affect the expression of resistance directed against the other.

Table 4 shows the results of a test wherein groups of mice were given sensitizing s.c. implants of only the fibrosarcoma or of both the fibrosarcoma and the mammary carcinoma s.c. at the right shoulder and hip. The effect of early or late radiotherapy or surgical removal of the sensitizing fibrosarcoma implants on the resistance against challenge with the fibrosarcoma was determined as in the previously described tests. In addition, 4 groups (Groups 2 to 5) in which the sensitizing fibrosarcoma implants were removed 4 days after removal of the sensitizing implants (see text).

Table 5 shows the results of a test designed to determine quantitatively the depressing effect of tumor tissue on tumor-specific host resistance. The mice were sensitized with

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of cells injected</th>
<th>No. of mice with tumors/ no. challenged</th>
<th>Total no. of tumors/no. challenged</th>
<th>Average tumor size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Surgery at -4 days</td>
<td>1 X 10⁶</td>
<td>7/8</td>
<td>10/16</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>Irradiation at -4 days</td>
<td>1 X 10⁶</td>
<td>6/6</td>
<td>10/12</td>
<td>10.5</td>
</tr>
<tr>
<td>3</td>
<td>Surgery at +4 days</td>
<td>1 X 10⁶</td>
<td>9/9</td>
<td>16/18</td>
<td>12.6</td>
</tr>
<tr>
<td>4</td>
<td>Irradiation at +4 days</td>
<td>1 X 10⁶</td>
<td>10/10</td>
<td>19/20</td>
<td>12.9</td>
</tr>
<tr>
<td>5</td>
<td>Unsensitized</td>
<td>3.3 x 10⁶</td>
<td>10/10</td>
<td>20/20</td>
<td>14.5</td>
</tr>
<tr>
<td>6</td>
<td>Unsensitized</td>
<td>1 X 10⁶</td>
<td>10/10</td>
<td>20/20</td>
<td>16.8</td>
</tr>
<tr>
<td>7</td>
<td>Unsensitized</td>
<td>3.3 x 10⁴</td>
<td>10/10</td>
<td>16/20</td>
<td>12.1</td>
</tr>
</tbody>
</table>

a The sensitizing tumors were implanted s.c. 19 days before treatment. Treatment consisted of surgical removal or lethal irradiation of the tumors 4 days before (-4 days) or 4 days after (+4 days) challenge implantation of a suspension of cells of the same tumor. Average tumor size at the time of treatment was 12 x 12 mm.

b See Footnote b, Table 1. Group 1 versus Groups 2 to 4 versus Group 6, p < 0.001.
### Table 4

The effect of a growing or irradiation-killed fibrosarcoma and mammary carcinoma on the resistance of the tumor host to fibrosarcoma challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Challenge</th>
<th>No. of cells injected</th>
<th>No. of mice with tumors/no. challenged</th>
<th>Total no. of tumors/no. challenged</th>
<th>Average tumor size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FS excised at -4 days</td>
<td>4/9</td>
<td>1 x 10^5</td>
<td></td>
<td>6/18</td>
<td>10.3</td>
</tr>
<tr>
<td>2</td>
<td>FS and MC excised at -4 days</td>
<td>6/10</td>
<td>1 x 10^6</td>
<td></td>
<td>7/20</td>
<td>8.4</td>
</tr>
<tr>
<td>3</td>
<td>FS and MC excised at -4 days</td>
<td>4/10</td>
<td>1 x 10^6</td>
<td></td>
<td>6/20</td>
<td>8.6</td>
</tr>
<tr>
<td>4</td>
<td>FS excised at -4 days; MC excised at +4 days</td>
<td>5/9</td>
<td>1 x 10^6</td>
<td></td>
<td>7/18</td>
<td>10.0</td>
</tr>
<tr>
<td>5</td>
<td>FS excised at -4 days; MC excised at +4 days</td>
<td>4/9</td>
<td>1 x 10^6</td>
<td></td>
<td>6/18</td>
<td>9.3</td>
</tr>
<tr>
<td>6</td>
<td>FS irradiated at -4 days</td>
<td>6/10</td>
<td>1 x 10^6</td>
<td></td>
<td>10/20</td>
<td>13.3</td>
</tr>
<tr>
<td>7</td>
<td>FS excised at +4 days</td>
<td>7/10</td>
<td>1 x 10^6</td>
<td></td>
<td>11/20</td>
<td>12.9</td>
</tr>
<tr>
<td>8</td>
<td>FS irradiated at +4 days</td>
<td>6/8</td>
<td>1 x 10^6</td>
<td></td>
<td>12/16</td>
<td>15.1</td>
</tr>
<tr>
<td>9</td>
<td>Unsensitized</td>
<td>10/10</td>
<td>3.3 x 10^6</td>
<td></td>
<td>20/20</td>
<td>18.2</td>
</tr>
<tr>
<td>10</td>
<td>Unsensitized</td>
<td>10/10</td>
<td>3.3 x 10^6</td>
<td></td>
<td>18/20</td>
<td>15.8</td>
</tr>
<tr>
<td>11</td>
<td>Unsensitized</td>
<td>10/10</td>
<td>3.3 x 10^6</td>
<td></td>
<td>14/20</td>
<td>9.8</td>
</tr>
</tbody>
</table>

The sensitizing tumors were implanted s.c. in the right flank 20 days before treatment (see text). FS, fibrosarcoma; MC, mammary carcinoma. See treatment, Table 2, Footnote a. The average size of the fibrosarcomas was 10 x 10 mm, and the average size of the mammary carcinomas was 12 x 11 mm at the time of removal.

b See Table 1, Footnote b. Groups 1 to 5 versus Groups 6 to 8 versus Group 10, p < 0.001

### Table 5

The effect of injection of killed fibrosarcoma cells and killed mammary carcinoma cells on the resistance of the tumor host to fibrosarcoma challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Challenge</th>
<th>No. of cells injected</th>
<th>Total no. of tumors/no. challenged</th>
<th>Average tumor size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FS excised at -4 days</td>
<td>1/9</td>
<td>1 x 10^4</td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>FS excised at -4 days, and 5 x 10^3 g</td>
<td>4/10</td>
<td>1 x 10^5</td>
<td></td>
<td>10.8</td>
</tr>
<tr>
<td>3</td>
<td>FS excised at -4 days, and 5 x 10^3 g</td>
<td>5/10</td>
<td>1 x 10^6</td>
<td></td>
<td>11.6</td>
</tr>
<tr>
<td>4</td>
<td>FS excised at -4 days, and 5 x 10^4 g</td>
<td>4/10</td>
<td>1 x 10^6</td>
<td></td>
<td>7.8</td>
</tr>
<tr>
<td>5</td>
<td>FS excised at -4 days, and 5 x 10^4 g</td>
<td>2/10</td>
<td>1 x 10^5</td>
<td></td>
<td>7.9</td>
</tr>
<tr>
<td>6</td>
<td>MC excised at -4 days, and 5 x 10^3 g</td>
<td>2/10</td>
<td>1 x 10^4</td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>7</td>
<td>MC excised at -4 days, and 5 x 10^3 g</td>
<td>1/10</td>
<td>1 x 10^4</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>8</td>
<td>MC excised at -4 days, and 5 x 10^4 g</td>
<td>0/10</td>
<td>1 x 10^5</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>9</td>
<td>MC excised at -4 days, and 1 x 10^4 g</td>
<td>0/10</td>
<td>1 x 10^6</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>MC irradiated at -4 days</td>
<td>4/8</td>
<td>1 x 10^5</td>
<td></td>
<td>13.0</td>
</tr>
<tr>
<td>11</td>
<td>FS excised at +4 days</td>
<td>4/10</td>
<td>1 x 10^5</td>
<td></td>
<td>10.3</td>
</tr>
<tr>
<td>12</td>
<td>FS irradiated at +4 days</td>
<td>5/9</td>
<td>1 x 10^5</td>
<td></td>
<td>8.8</td>
</tr>
<tr>
<td>13</td>
<td>Unsensitized</td>
<td>10/10</td>
<td>3.3 x 10^5</td>
<td></td>
<td>16.8</td>
</tr>
<tr>
<td>14</td>
<td>Unsensitized</td>
<td>10/10</td>
<td>3.3 x 10^5</td>
<td></td>
<td>15.8</td>
</tr>
<tr>
<td>15</td>
<td>Unsensitized</td>
<td>4/10</td>
<td>3.3 x 10^5</td>
<td></td>
<td>9.0</td>
</tr>
</tbody>
</table>

The sensitizing tumors were implanted s.c. in the right flank 21 days before treatment (see text). FS, fibrosarcoma; MC, mammary carcinoma. For description of treatment, see text. Average tumor size at the time of treatment was 10 x 10 mm.

b All mice were challenged with one s.c. injection of tumor cells in the left flank. Group 1 versus Groups 10 to 12 versus 14, p < 0.001; Groups 6 to 9 versus groups 2 to 5 versus Group 14, p < 0.001.
s.c. implants of living pieces of fibrosarcoma. Groups 1, 10, 11, and 12 were treated with extirpation or irradiation of the sensitizing implants either 4 days before or 4 days after challenge. In addition, Groups 2, 3, 4, and 5 were given s.c. injections 3 times with graded doses of 0.9% NaCl solution suspensions of irradiation-killed fibrosarcoma cells. Injections were given at the time of surgical removal of the sensitizing tumor 4 days before challenge, again at the time of challenge, and 4 days after challenge. Groups 6, 7, 8, and 9 were given a similar course of treatments, but they were given injections of graded doses of suspensions of irradiation-killed mammary carcinoma cells. All injections were given in a volume of 0.05 ml.

The results show that at the 3 lowest doses only the injections of fibrosarcoma cells depressed the resistance of the hosts to challenge implants of the fibrosarcoma tumor. At the highest dose, the injected mammary carcinoma cells also appear to have depressed host resistance, but the effect, if real, appears to be slight, and the figures for Group 6 do not differ markedly from those of Group 1. The effects of residual or injected tumor tissue are expressed in the number of mice that developed tumors following challenge, as well as in the average tumor size. The lower limit of specific depression of resistance by injections of killed tumor cells appears to have been approached but not reached with the 100-μg dose of fibrosarcoma cells. The differences in tumor incidence between untreated control mice, mice with residual or injected fibrosarcoma tissue, and mice surgically cured and free of fibrosarcoma tissue were statistically significant.

DISCUSSION

The question concerning the existence of a state of concomitant immunity in a tumor host is essentially one of whether an antigenic tumor mass consumes or neutralizes specific host-immune factors to an extent which may leave the host without resistance against the growth of injected or naturally disseminated tumor cells.

The results of this investigation show that the antitumor resistance of the tumor hosts was active in spite of—but was depressed by—the presence of an antigenic fibrosarcoma mass. Their resistance was also depressed by s.c. injections of killed fibrosarcoma cells. The depression was specific, since a growing mammary carcinoma (Table 4) or injected mammary carcinoma cells (Table 5) had little or no effect on the growth of fibrosarcoma implants. The depression was dose related, as shown by the greater degree of depression with the injection of the highest doses of fibrosarcoma cells (Table 5). The apparent depression by the highest dose of mammary carcinoma cells is insignificant in comparison with the values of Group 1, but it may indicate a weak, nonspecific depression of resistance.

The observed depression of resistance resembles most closely the immunological phenomenon of desensitization, which refers to the ability of antigen to cause a temporary depression of existing hypersensitivity of the immediate or delayed types. The temporary nature of the depression seen here is indicated by the increase in resistance to tumor challenge at 4 days after surgical removal of the sensitizing tumor implants.

Specific desensitization by s.c. injections of antigen (hyporesponsiveness) is a common clinical procedure used to relieve allergic conditions of the immediate type, such as hay fever and asthma (25).

Specific desensitization of delayed hypersensitivity has not been widely reported, but it has been observed by Uhr and Pappenheimer (33), who found that administration of large doses of the specific antigen resulted in desensitization of guinea pigs hypersensitive to ovalbumin. Leskowitz and Waksman (17) made similar observations in rabbits with delayed hypersensitivity to bovine serum albumin. Leskowitz and Jones (16) demonstrated hapten-specific desensitization of guinea pigs with delayed hypersensitivity to arsanil-conjugated bovine serum albumin.

The observations reported here resemble those reported by Wepsic et al. (39), who found that the adoptive transfer of resistance to a syngeneic diethylaminoiminoconjugated guinea pig hepatoma was specifically inhibited by the presence in the recipients of live or killed tumor cells or by the in vitro incubation of the immune effector cells with tumor cells before adoptive transfer.

Immunological enhancement of tumor growth cannot be excluded as a factor in the depression of host resistance by residual or injected tumor tissue. It is known from experimental and clinical investigations that enhancing or "blocking" antibodies can occur in human patients and in animal tumor hosts at the same time as a state of resistance manifested by the action of lymphoid cells against tumor cells in vitro (8, 12, 23, 40). It is possible that the expression of enhancement or resistance against a tumor is an expression of the relative balance of the 2 factors (12) and that the enhancing factors are favored by the presence of large quantities of tumor antigens.

The rapid change in resistance seen here after removal of the sensitizing tumor indicates recovery from desensitization, rather than loss of antibody-mediated enhancement.

In some reports of investigations, where resistance factors could not be detected in hosts carrying a sensitizing tumor, the explanation was offered that an antigenic tumor mass may act in a manner analogous to the action of a sponge. By antigenic attraction and binding, the specific resistance factors are absorbed as soon as they are formed and cannot accumulate in detectable amounts until the antigenic mass has been removed. This suggestion is not supported by the experimental evidence from transfer studies with sensitized cells labeled with thymidine-3H (18, 32) in which it was shown that sensitized cells were not specifically attracted to antigenic depots but that the large majority of the cells involved in delayed hypersensitivity reactions were unsensitized cells of host origin. Therefore, it seems more likely that the depression reported here was effected systemically by antigens released into immature animals. The term "immune paralysis" is now used interchangeably with "tolerance," but in a stricter sense it describes a state of unresponsiveness produced in mature animals by the injection of excessively large amounts of antigen to preclude an immune response. Tumor immunity, being mainly cell mediated, comes under the broad heading of delayed hypersensitivity.
from the tumor mass or dispersed from the s.c. depot of injected, killed tumor cells.

The mechanism of desensitization may act in at least 2 different ways. First, the antigen may specifically destroy sensitive effector cells, or second, the antigen may bind the specific reactive sites on sensitized cells to make them incapable of further interaction with antigen.

The 1st mechanism is contradicted by experimental evidence. Studies of cellular immune responses in vitro have shown that specifically sensitized lymphocytes do not appear to be damaged by exposure to antigen, but respond by incorporation of thymidine-\(^3\)H (6 21) and blast cell transformation (22), and by the synthesis of biologically active substances (4). The 2nd mechanism is supported by the data reported by Asherson and Stone (1), who inhibited the adoptive transfer of delayed hypersensitivity to bovine \(\gamma\)-globulin by exposing sensitized guinea pig peritoneal exudate cells to the specific antigen for 20 min before transfer. The brief period of exposure needed to desensitize the cells was regarded as an indication that the factors affected were located on the cell surface.

The results of the present investigation pertain to a particularly important question in tumor immunology. In the event of clinical immunotherapy of cancer, when may it be considered safe and beneficial to inject tumor tissue preparations as autovaccines into patients? The present data indicate that a critical factor in host resistance may well be the amount of free antigen present in the host at any time. In the proper amount, tumor antigen will initiate and support an immune response, but when this amount is exceeded, as in the tests reported here, the resistance factors become neutralized. Accordingly, in a case where more than minimal residual tumor tissue is suspected or known to exist, injection of additional tumor antigen in the form of a nonviable autovaccine preparation could be excessive and could result in a temporary depression of an existing immune state. More information is needed in this area and should be derived from experimentation in animal systems before specific immunotherapy may be tested in clinical trials.

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REFERENCES


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