Resistance of Tumor-bearing Mice to a Second Tumor Challenge

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ABSTRACT

Antitumor resistance of mice bearing 3LL, M109, or Meth A tumors to a second tumor challenge was studied. The degree of inhibition of a second tumor growth in the tumor-bearing mice was a function of both the size of the primary tumor mass and the number of reinoculated tumor cells.

Antitumor resistance of tumor-bearing mice is tumor nonspecific and is observed only in the presence of the growing first tumor. Following removal of 3LL tumor, the resistance to the second challenge with 3LL or T-10 tumor cells completely disappeared.

Antitumor resistance of tumor-bearing mice does not appear to be mediated by T-cells, natural killer cells, or macrophages, since similar results were observed in immunologically competent and nude mice as well as in beige mice and in mice with functions of macrophages and natural killer cells suppressed by silica treatment. Growth inhibition of the reimplanted tumor cells does not appear to be a result of their elimination. Radiolabeled M109 tumor cells survived to the same degree at the local site of inoculation in tumor-bearing and non-tumor-bearing control mice. The number of surviving radiolabeled tumor cells inoculated into the footpad of immunocompetent and nude mice was similar and did not depend on the presence or absence of growing first tumor. Despite the similarity in the survival of reimplanted tumor cells, their growth was inhibited only in the tumor-bearing mice. In mice bearing immunogenic Meth A tumors of different sizes (1 to 3 cm), the clearance of the reimplanted Meth A cells was significantly higher than in non-tumor-bearing mice. The number of surviving tumor cells was similar in mice bearing small (<1 cm) or large (>3 cm) tumors. However, complete prevention of the second tumor growth was observed in mice bearing huge tumor mass (>3 cm in diameter). These data may indicate that resistance of mice bearing immunogenic and nonimmunogenic tumors is mediated by different mechanisms. The resistance to a second tumor challenge in mice bearing nonimmunogenic tumor is due mainly to nonimmunological mechanisms. In contrast, the antitumor resistance in mice bearing immunogenic tumor is a function of antitumor immune reactions, although additional effects of nonimmunological mechanisms were also observed.

INTRODUCTION

The inhibition of growth of a second tumor graft in mice bearing the original tumor was described by Ehrlich (6) in 1906. This phenomenon was later attributed to the antitumor immunological response and termed concomitant tumor immunity (2). Although the antitumor resistance of tumor-bearing mice to a second challenge is rather strong, even at high doses of tumor cell inocula, this experimental model did not attract much attention. It required about 50 years to prove that the specific immune response can be evoked during tumor growth, resulting in increased resistance to the second tumor challenge (23). In this experimental model, the antitumor resistance of the host was analyzed after removal of the first tumor graft. The antitumor resistance in the animals following excision of the growing tumor became a favorite model for measuring the antitumor immune response, immunogenicity, and the antigen specificity of the investigated tumors (18, 21, 23). In 1970, Fisher et al. (7) proposed to distinguish concomitant immunity (resistance to a second tumor graft in the presence of the first growing tumor) from sinecomitant immunity (resistance of tumor-excised animals to a second challenge). In many respects, the resistance to a second tumor challenge shown by the tumor-excised host has different characteristics from the resistance observed in the tumor-bearing host.

In previous studies (15), we have found that the resistance of mice to a second tumor graft in the presence of the growing tumor can be very strong and may completely prevent the growth of high doses of the reinoculated tumor cells. This resistance was a function of the volume of the primary tumor mass and was not tumor specific. Indeed, mice bearing 3LL tumors were resistant to the reinoculation of 3LL, B16, or EL4 tumors. Similarly, the growth of 3LL tumor cells was arrested when they were reinoculated into mice bearing B16, EL4, or T-10 tumors. It appears that this resistance is not mediated via T-cell-mediated immunity because in T-cell-depleted animals (B-mice or nude mice) bearing the first tumor graft, the inhibitory effect on the subsequently implanted tumor cells was as effective as in normal tumor-bearing mice (15).

The tumor-nonspecific character of the resistance to a second tumor challenge and its independence from T-cell-mediated immunity suggest that either NK cells or macrophages may be responsible for the inhibition of a second tumor growth. In the present study, the possible involvement of NK cells and macrophages in the resistance of the tumor-bearing host to a second tumor challenge was investigated. Inhibition of tumor growth can result from elimination of the tumor cells or prevention of their proliferation. To analyze the survival and growth of tumor cells reinoculated into normal or tumor-bearing mice, we used a new radioisotope technique that makes it possible to determine the survival of tumor cells at the inoculation site (11, 12).

MATERIALS AND METHODS

Mice. Inbred female C57BL/6-H-2², beige C57BL/6-H-2², and BALB/c-H-2² mice, 2 to 3 months old, were obtained from The Jackson Laboratory, Bar Harbor, Maine. (C3H/eB × C57BL/6) (H-2²/H-2²) F₁ mice were supplied by the Animal Breeding Center of the Weizmann Institute of Science, Rehovot, Israel.

Tumors. The following tumors were used: Lewis lung carcinoma

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² The abbreviations used are: NK, natural killer; dUrd, deoxyuridine; f.l.p., into the footpad.
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(3LL) that arose spontaneously in C57BL/6 mice; Meth A fibrosarcoma induced by methylcholanthrene in BALB/c mice; Madison lung carcinoma (M109) spontaneously developed in BALB/c mice; YAC-1, a tissue culture cell line of YAC, a Moloney virus-induced lymphoma of A/J origin. These tumor cells were maintained in vitro in complete Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal bovine serum (11, 12). The T-10 tumor was induced by methylcholanthrene in (C3H/eB × C57BL/6) F1 mice and maintained by serial transfers in these mice.

Tumor Cell Survival In Vivo. Nonconfluent monolayers of cultivated M109, Meth A cells, or nonsaturated cultures of YAC-1 lymphoma cells were incubated for 16 h with fluorouridine (0.06 mg/ml) and 125I-DUrd (0.4 μCi/ml; specific activity, 5 Ci/mg; The Radiochemical Centre, Amersham, England). After washing, tumor cells were resuspended in complete Roswell Park Memorial Institute Medium 1640. Radiolabeled tumor cells were inoculated i.f.p. in normal or tumor-bearing mice. The level of radioactivity in the footpad was measured at 30 min and then at various times following tumor cell inoculation. The level of radioactivity in the inoculated leg was measured with a low-energy γ-scintillation counter (Ludlum model 2200; Ludlum Measurements Inc., Sweetwater, Texas) adjusted to detect γ-rays between 10 and 40 keV, using a detector with a 1-mm-thick, 1-inch-diameter NaI (T1) crystal with a 7 mg per sq cm aluminum window. The detector was shielded with 2-mm-thick lead with an aperture of 1 x 1.5 cm. The inoculated foot was put into this opening, and the level of the radioactivity was measured during 20 sec. Mice were marked, making it possible to follow the rate of clearance of radioactivity in individual mice. Vernier calipers were used to measure the diameter of i.f.p. established tumors at various periods of growth.

In Vitro Cytotoxic Activity of Spleen Cells. Cytotoxic effect of spleen cells of normal or tumor-bearing mice was assessed in a 4-hr assay of 51Cr release as described previously (11). Some of the control or tumor-bearing mice were treated with 15 mg of silica (Sanocel 58; particle size 3 μm; Monsanto Co., St. Louis, Mo.) 3 days before the cytotoxic activity of their spleen cells was tested.

RESULTS

In the first series of experiments, the growth of a second tumor graft was studied in tumor-bearing mice and in mice from which primary tumors had been removed. In these studies, there are certain difficulties concerning the survival of tumor-bearing mice. Relatively small doses of reinoculated tumor cells can have a long latent period, and tumor-bearing mice can die before the tumors appear even in the control mice. High doses of reinoculated tumor cells may overcome the antitumor resistance developed in tumor-bearing and tumor-excised mice. Our previous experiments had shown that (C3H/eB × C57BL/6) F1 mice can tolerate the 3LL tumors at the size that usually kills syngeneic C57BL/6 mice. Thus, F1 mice were chosen for these experiments. It was found previously that mice bearing s.c. tumors larger than 1 cm in diameter had apparent resistance to the second tumor graft (15).

To facilitate the excision of the local tumor, 1 x 10^6 3LL tumor cells were inoculated i.f.p. into (C3H/eB × C57BL/6) F1 mice. When tumors reached 10 to 12 mm, mice were divided into 2 groups. In the first group, tumor-bearing legs were amputated; in the second group, tumors were allowed to grow. Tumor-bearing and tumor-excised mice were reinoculated in the second leg with 1 x 10^6 3LL or T-10 tumor cells on the day of surgery. Control mice were inoculated i.f.p. with the same doses of tumor cells.

Most of the mice bearing the first tumor survived at least 8 days after tumor reinoculation, which allowed comparison of the tumor growth of reimplanted tumor cells in the presence and absence of the first tumor graft. Chart 1 shows the tumor diameter in the reinoculated leg. The diameters of 3LL tumors from reinoculated cells were similar in the tumor-excised and control mice whereas in mice bearing the first 3LL tumor the growth of the second 3LL tumor was substantially suppressed.

This inhibition was not tumor specific because growth of reinoculated T-10 tumor cells was inhibited in the presence of growing 3LL tumor. In contrast, there was no inhibition of T-10 tumor growth in mice that had had excision of 3LL tumor (Chart 1). Previous experiments (15) have demonstrated that growth of the reinoculated tumor cells was effectively prevented in the tumor-bearing T-cell-deficient animals (B-mice or nude mice). Antitumor resistance of tumor-bearing mice with either intact or deficient T-cell-mediated immunity could result from the cytotoxic action of NK cells or macrophages, which have cytotoxic action that is not tumor specific.

This assumption was analyzed using mice in which the functions of NK cells and macrophages were suppressed (C57BL/6 mice treated with silica; beige mice). Beige mice are characterized by a low level of NK cell activity, and silica treatment suppresses the functions of both NK cells and macrophages (1, 5). C57BL/6 and beige mice were inoculated s.c. with 1 x 10^6 3LL tumor cells. When tumors reached 2 cm in diameter, some of the tumor-bearing C57BL/6 mice and some of the control C57BL/6 mice were inoculated i.p. with 15 mg of silica. All mice were inoculated i.f.p. with 1 x 10^6 3LL tumor cells 24 hr later. Tumor growth i.f.p. was similar in silica-treated, control C57BL/6 mice and in beige mice. However, in all groups of mice bearing s.c. tumors, the growth of the second tumor graft was strongly inhibited (Chart 2). Peritoneal macrophages of normal or silica-treated tumor-bearing mice had no cytotoxic activity against tumor cells assessed by in vitro technique (data not shown).

The activity of NK cells in the tumor-bearing mice was tested in vitro using a 4-hr assay of 51Cr release (Table 1). In a series of experiments, the cytotoxic effect of spleen cells of intact C57BL/6 mice at an effector:target ratio of 100:1 varied from 12 to 19%, whereas spleen cells of mice bearing 3LL tumor >2 cm in diameter had lower cytotoxic activity which never exceeded 6%. Cytotoxic activity in the silica-treated mice was almost completely suppressed.

Natural cell-mediated immunity also can be assessed in vivo by the ability of mice to eliminate radiolabeled tumor cells
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Previously, we have shown that M109 tumor cells resist the cytotoxic action of NK cells in vitro and their survival in vivo were similar in mice with low and high NK cell activity. Tumor growth was observed in syngeneic BALB/c+ +/+ and BALB/c nude mice inoculated with radiolabeled M109 tumor cells (11, 12).

The levels of radioactivity immediately after the inoculation reflect the real number of injected tumor cells (Chart 4A). Measurement of the radioactivity in the foot pads at different times after tumor cell inoculation indicates the level of tumor cell survival. One day after tumor cell inoculation, a decrease in radioactivity was observed in all mice, but later the rate of elimination of radioactivity from the footpad decreased. The levels of radioactivity detected in normal and tumor-bearing BALB/c mice were similar. In addition, no differences were found in the clearance of radioactivity in BALB/c+ +/+ and nude mice bearing s.c. tumors and in non-tumor-bearing mice. The absolute levels of radioactivity in the footpads of nude control mice were lower than in other mice, but this was a result of the initial lower number of radiolabeled cells inoculated i.f.p. in these mice (Chart 4A). In general, the slope of the elimination of radioactivity in nude mice was identical to the slope for other BALB/c mice.

The similarity in the levels of radioactivity remaining in the inoculated i.v. from the lungs (24) or from the inoculation site (11). The ability of normal or tumor-bearing C57BL/6 mice to eliminate 125I-dUrd-labeled YAC-1 cells from the footpad was studied; 0.5 × 10⁶ radiolabeled YAC-1 cells were inoculated i.f.p. in control C57BL/6 mice or mice bearing s.c. 3LL tumors of different sizes (≤1, ≥2, or ≥3 cm). The level of radioactivity remaining in the leg was determined.

In C57BL/6 mice bearing 3LL tumors ≥3 cm, the elimination of tumor cells from the inoculation site was substantially suppressed (Chart 3). This suppression was less in mice bearing smaller 3LL tumors (2 to 3 cm in diameter). In mice bearing the small s.c. 3LL tumors (≤1 cm), the clearance of reinoculated YAC-1 tumor cells was significantly greater than in other tumor-bearing mice and was similar to the clearance in non-tumor-bearing control mice. These experiments indicate that cytotoxic activity of NK cells tested in vivo and in vitro against NK-susceptible tumor cells is impaired in tumor-bearing mice.

Survival and growth of reinoculated radiolabeled tumor cells can be a useful method for understanding the mechanism of the tumor growth suppression in tumor-bearing mice. It is still unclear whether the growth inhibition of the second tumor graft results from the elimination of the reinoculated tumor cells or from the suppression of their proliferation. To make this determination, BALB/c mice and BALB/c nude mice were inoculated s.c. with 1 × 10⁶ M109 tumor cells. When s.c. tumors reached ≥2 cm in diameter, mice were inoculated i.f.p. with 2 × 10⁶ [125I]-dUrd-labeled M109 tumor cells. Control BALB/c mice, BALB/c nude mice, and allogeneic C57BL/6 and CBA/J mice received the same number of radiolabeled M109 tumor cells. Previously, we have shown that M109 tumor cells resistant to the cytotoxic action of NK cells in vitro and their survival.

**Table 1**

<table>
<thead>
<tr>
<th>Mice</th>
<th>% of cytotoxicity</th>
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<tr>
<td>100:1⁴</td>
<td>33:1</td>
</tr>
<tr>
<td>11:1</td>
<td></td>
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<tr>
<td>Intact</td>
<td>15.7 ± 0.9</td>
</tr>
<tr>
<td>Silica-treated non-tumor-bearing</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Tumor-bearing</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>Silica-treated tumor-bearing</td>
<td>3.9 ± 0.1</td>
</tr>
</tbody>
</table>

Effect:target ratio. Mean ± S.E. Significantly different (p < 0.01) from other groups.

Chart 3. Elimination of radiolabeled YAC-1 cells from footpad of control mice or mice bearing s.c. 3LL tumor of different sizes. Control C57BL/6 mice or mice bearing s.c. 3LL tumors (≤1, ≥2, or ≥3 cm in diameter) were inoculated i.f.p. with 0.5 × 10⁶ [125I]-dUrd-labeled YAC-1 cells. Level of remaining radioactivity i.f.p. was measured at various periods after tumor cell inoculation (5 mice/group). Bars, S.E.
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Chart 4. Elimination of radiolabeled M109 tumor cells from footpad of control and tumor-bearing mice. In A, control syngeneic BALB/c-+/ + (○), BALB/c nude (Δ), allogeneic C57BL/6 (●), and CBA/J (□) mice were inoculated i.f.p. with 2 × 10^6 [131]I-dUrd-labeled M109 tumor cells. Radiolabeled M109 tumor cells (2 × 10^6) were also reinoculated i.f.p. in BALB/c-+/+ (●) or BALB/c nude mice (Δ) bearing s.c. M109 tumor ≥2 cm in diameter. At various periods after inoculation of radiolabeled cells, the level of radioactivity remaining i.f.p. was measured. In B, control CBA/J mice (□), BALB/c-+/+ mice (○), or BALB/c-+/+ mice bearing s.c. M109 tumor ≥2 cm (●) were inoculated i.f.p. with 1 × 10^6 radiolabeled M109 tumor cells (10 mice/group).

foot pads of BALB/c-+/+ and nude mice bearing the first tumor graft and of non-tumor-bearing mice may indicate: (a) that cytotoxic T-cells did not develop in BALB/c-+/+ mice; or (b) that NK cells, macrophages, or other potential cytotoxic agents were not active in all mice investigated or that they were equally efficient in tumor-bearing and non-tumor-bearing mice and in immunocompetent and T-cell-depleted nude mice. The potential efficiency of immune reaction in the elimination of inoculated radiolabeled M109 tumor cells was indicated by the elimination of these cells in allogeneic C57BL/6 and CBA/J mice. Seven days after inoculation of radiolabeled cells, the slope of radioactivity in allogeneic and syngeneic mice was identical (Chart 4A). Later, however, the rate of clearance of tumor cells rapidly increased in the allogeneic mice and, 13 days after tumor cells were injected, all radioactivity in the allogeneic mice had completely disappeared (Chart 4A). The rate of clearance of M109 tumor cells was slightly higher in C57BL/6 mice than in CBA/J mice. Although the levels of radioactivity were similar in the footpads of BALB/c-+/+ and nude mice bearing s.c. tumors and in non-tumor-bearing mice, the rates of tumor cell growth in these mice were different (Chart 4A). In intact BALB/c-+/+ and BALB/c nude mice, i.f.p. tumors grew progressively, but the growth of reinoculated M109 tumor cells was strongly suppressed in mice bearing s.c. M109 tumor. In the presence of the first tumor, this suppression was even more profound in nude mice than in BALB/c-+/+ mice (Chart 4A). Nude mice bearing s.c. M109 tumor appeared to be very ill. They lost weight and were pale and less active than nude mice that had no s.c. tumors. Similar results were obtained when, in parallel, BALB/c mice bearing s.c. M109 tumors (≥2 cm in diameter) were reinoculated i.f.p. with a higher dose of radiolabeled M109 tumor cells (1 × 10^6). The slope of radioactivity in the inoculated legs of tumor-bearing and control BALB/c mice was similar (Chart 4B). Fourteen days after reinoculation of tumor cells, a high level of radioactivity remained at the site of transplantation, although a slight decrease in radioactivity was found in BALB/c mice not bearing s.c. tumors. At this period, these mice developed i.f.p. tumors that were >0.5 cm in diameter, which made it difficult to measure the level of radioactivity in the leg. In allogeneic CBA/J mice inoculated with radiolabeled M9 tumor cells, the tumor cells were completely eliminated (Chart 4B). In contrast to the similarity in the survival of reinoculated M109 cells in tumor-bearing and control BALB/c mice, there was a striking difference in the growth of reinoculated tumor cells (Chart 5B). In the presence of s.c. M109 tumor, the proliferation of the reinoculated M109 tumor was strongly suppressed.

All of these experiments were performed using weakly immunogenic spontaneous 3LL and M109 tumors. To assess the contribution of the immunological mechanisms in the elimination of reinoculated tumor cells and in the suppression of their growth in tumor-bearing mice, the immunogenic methylcholanthrene-induced Meth A tumor was used. The antigenic and immunogenic properties of Meth A tumor have been reported in numerous investigations (3, 21, 25). BALB/c mice were inoculated s.c. with 1 × 10^6 Meth A tumor cells. Mice bearing s.c. Meth A tumors that were ≥3, ≥2, or ≥1 cm in diameter were inoculated i.f.p. with 0.8 × 10^6 125I-dUrd-labeled Meth A tumor cells. The level of radioactivity at the inoculation site progressively decreased in all mice. However, mice bearing s.c. Meth A tumors had more efficient clearance of radioactive tumor cells than did normal non-tumor-bearing BALB/c mice inoculated i.f.p. with radioactive Meth A tumor cells (Chart 6). Although the levels of radioactivity remaining in the footpads of all tumor-bearing mice were similar, large differences were observed in the growth rates of reinoculated tumor cells (Chart 7). In mice bearing tumors ≥3 cm, growth of i.f.p. reinoculated tumor cells was completely suppressed. There was substantial inhibition in the development of second-footpad tumors in mice bearing first s.c. tumors ≥2 cm in diameter. Fourteen days
after reinoculation of Meth A cells i.f.p., visible tumors developed in 3 of 9 surviving mice. All mice had symptoms of cachexia. However, tumor-bearing mice in which growth of the second tumor graft i.f.p. was completely prevented had more profound symptoms of cachexia and loss of activity than mice in which some growth of i.f.p. tumor was observed; 15 to 19 days after i.f.p. reinoculation of radiolabeled Meth A cells, 6 of the 9 mice were dead. None of them had established tumors in the foot. Tumors developed in the 3 surviving mice, although tumor size was smaller than in control mice (Chart 7). In mice that had s.c. tumors ≥1 cm at the time of i.f.p. reinoculation with Meth A cells, the appearance and growth of tumors were similar to those in control mice. On Day 12 after reinoculation of tumor cells, the first s.c. tumors increased in size and after Day 12 growth retardation of i.f.p. tumors was observed. On Day 19 after reinoculation, substantial differences in the size of i.f.p. tumors were found in these and in control mice. In general, suppression of growth of reinoculated tumor cells in mice bearing small s.c. tumors was less profound than in mice bearing large s.c. tumors (Chart 7).
DISCUSSION

Resistance of mice to a second tumor graft in the presence of the growing primary tumor or after its excision is thought to be mediated by immunological mechanisms, concomitant and sinecomitant immunity, respectively (7). However, these types of immunity have different characteristics (Table 2). In general, sinecomitant immunity was observed when the removed tumors were relatively small and the number of cells used for the second challenge was very small. Larger numbers of reinoculated tumor cells can overcome the antitumor resistance of these animals. Antitumor resistance was observed when tumor reexcision was performed 7 to 10 days after removal of the first tumor. This period was probably required for the restoration of immune functions suppressed by the growing tumor (17–19, 22). In the present study as well as in the previous study (15), we have found that tumor-bearing mice had a high level of resistance to the second graft, even when relatively high doses of tumor cells were reinoculated. This resistance can be eliminated by simple excision of the first tumor; 1 × 10^6 3LL tumor cells inoculated into tumor-excised mice grew at the same rate as in the control mice, whereas their growth was inhibited in the presence of the first tumor. Similar results were obtained by DeWys (4) and Kerney and Nelson (17).

Antitumor resistance may disappear after tumor excision because the continuing presence of antigenic material is necessary to maintain antitumor immunity (8). However, excision of tumor does not mean that all antigenic material was removed, because 3LL tumor is a metastasizing tumor and at the time of excision metastatic cells have spread and settled in the lungs (13, 14). In addition, 1 × 10^6 3LL tumor cells were reinoculated i.f.p. These data may support the assumption that the inhibition of growth of the second graft was mostly mediated by the first tumor mass, with little or no involvement of the host immune system. Furthermore, antitumor sinecomitant immunity has been observed in immunologically competent hosts after excision of immunogenic tumors (21, 23). Resistance to a second tumor challenge in tumor-bearing mice was observed against both immunogenic and nonimmunogenic tumors. Thus, strong inhibition was found when weakly immunogenic 3LL, B16, or M109 tumor cells were reinoculated into tumor-bearing mice. In addition, this antitumor resistance of tumor-bearing mice is not tumor specific. Mice bearing a 3LL tumor had inhibited growth of reinoculated antigenic non-cross-reactive B16 or EL4 tumors, and mice bearing B16, EL4, or T10 tumors had arrested growth of reinoculated 3LL tumor cells (15). The nonspecific character of the inhibition of rechallenge tumor cells has been observed by several investigators (17, 20).

**Table 2**

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<thead>
<tr>
<th>Characteristics</th>
<th>Tumor-excised mice</th>
<th>Tumor-bearing mice</th>
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<tbody>
<tr>
<td>Ability to inhibit the growth of a second tumor graft</td>
<td>Weak</td>
<td>Strong</td>
</tr>
<tr>
<td>Immunogenicity of reinoculated tumor cells</td>
<td>Only immunogenic</td>
<td>Immunogenic and nonimmunogenic</td>
</tr>
<tr>
<td>Antigen specificity of tumor cells</td>
<td>Specific</td>
<td>Can be nonspecific</td>
</tr>
<tr>
<td>Host</td>
<td>Immunocompetent</td>
<td>Can be immunosuppressed or immunodeficient</td>
</tr>
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</table>

Inhibition of the growth of the second tumor graft was observed in the immunologically deficient mice. The growth of reinoculated tumor cells was equally inhibited in tumor-bearing B-mice and nude mice as well as immunologically competent mice (15). These results may indicate that the observed antitumor resistance of tumor-bearing mice is not mediated by T-cells. As an alternative, NK cells and macrophages could be considered as possible effector cells that participate in the rejection of the second tumor graft. This seems unlikely, however, because it has been shown in many experiments that, at the latest stages of tumor growth, the functions of the immune system, including those of NK cells and macrophages, are suppressed (9, 16, 19, 22). The present study supports the findings that NK cell function, which was tested in vitro and in vivo, was strongly suppressed in mice bearing 3LL tumors 2 to 3 cm in diameter. The growth of reinoculated 3LL tumor cells was strongly suppressed in silica-treated or in NK-deficient beige mice bearing s.c. 3LL tumors, indicating that NK cells and macrophages cannot be considered to be effector cells responsible for the rejection of tumor cells reinoculated into tumor-bearing mice.

Radioisotope technique showed that radiolabeled, NK-resistant M109 tumor cells reinoculated into tumor-bearing mice were not eliminated but survived at the inoculation site to the same degree as in non-tumor-bearing mice. The same results were obtained when radiolabeled cells were inoculated into control and tumor-bearing nude mice. It is remarkable that survival of reinoculated, nonimmunogenic M109 tumor cells was similar in BALB/c mice and in BALB/c nude mice. When CBA/J and C57BL/6 mice were inoculated with allogeneic M109 tumor cells, these cells were quickly eliminated. Although reinoculated M109 tumor cells survived in syngeneic mice, their proliferation was strongly suppressed in BALB/c mice and in nude mice in the presence of large M109 tumors. These results also indicate that data on survival of tumor cells are not sufficient to predict the development and growth rate of subsequent tumors. Cytostatic mechanisms may suppress tumor cell proliferation and keep these cells in the dormant state or inhibit their growth. By inoculation of radiolabeled immunogenic Meth A tumor cells into tumor-bearing mice, it was found that their elimination was greater than in control mice. These differences in the elimination of reinoculated Meth A tumor cells can be explained by the participation of immune reaction developed by the host during growth of the first Meth A tumor. This reaction was not sufficient to destroy all reinoculated cells as it was observed in allogeneic CBA/J mice. The levels of survival of reinoculated Meth A tumor cells were similar in mice bearing small and large tumors. However, complete prevention of tumor growth was observed only in mice bearing large tumors. In mice bearing small tumors, growth of reinoculated Meth A cells was partially suppressed.

These data give the experimental basis for the conclusion that inhibition of the growth of the second tumor graft in tumor-bearing mice is mostly mediated by nonimmunological mechanisms. However, immune response developed during the growth of immunogenic tumor cells may have some additional effect on the elimination of reinoculated tumor cells and the suppression of their growth.

In experiments performed by North and Kirstein (20), mice bearing Sal tumor had greater resistance to the reinoculation of identical Sal tumor than to non-cross-reactive BP3 or MC5.
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tumor cells. Lymphocytes of mice bearing Sal tumor were specifically cytotoxic against Sal tumor cells (20). In thymectomized, lethally irradiated, and bone marrow-reconstituted B-mice bearing Meth A or Sal tumor, resistance to a second tumor challenge substantially decreased. However, some antitumor resistance remained; hence, the growth of a second graft in B-mice was significantly inhibited (3, 20).

These data may support the hypothesis that immunologically specific and nonspecific factors can be involved in the inhibition of the second tumor growth. Specific inhibition became apparent in the presence of specific immune response evoked against immunogenic tumor cells. The hypothesis that concomitant tumor immunity is responsible for the resistance of the tumor-bearing host to a second tumor challenge was based on the data obtained by Bashford et al. (2) using outbred mice and allogeneic tumors. This assumption was supported when allogeneic lymphoma in hamsters and syngeneic, strongly immunogenic tumors in mice were used (3, 7, 8, 17, 20). These tumors induced strong immune response but grew progressively. However, reinoculated tumor cells can be eliminated by developed immune reactions.

The mechanisms involved in the nonimmunological suppression of growth of a second tumor in the host bearing a nonimmunogenic tumor are mostly unknown. Ehrlich (6) explained the antitumor resistance of tumor-bearing mice by developing the hypothesis of athetic immunity. According to this hypothesis, the resistance to the second tumor graft is a result of the depletion in the tumor-bearing host of essential nutritional substances that are required for the growth of the second tumor. In mice bearing large tumors, cachexia and other symptoms of malignant disease were more profound, and inhibition of the growth of the second tumor graft was greater. Weight loss, which is common for a tumor-bearing organism, can be modulated in normal mice by food restriction. Nutrient restriction may be accompanied by strong inhibition of the development of spontaneous or induced tumors as well as transplanted tumors (10, 28). Since cachexia and other symptoms of malignant disease and resistance to the challenge of the second nonimmunogenic tumor usually disappeared after removal of the tumor, it is possible that the growing tumor is responsible for these phenomena.

Numerous data indicate that increase in tumor mass in the tumor-bearing organism takes place at the expense of the host tissues and is accompanied by severe metabolic disorders, impairment of enzyme function in the host tissues, and disturbance of endocrine regulation. No tissue functions normally in the tumor-bearing organism (26), and it appears that these conditions are less favorable to the proliferation of reinoculated tumor cells than are those in control animals.

It was also suggested that the tumor produces some factors with antiproliferative activity (4, 27, 29). The nature of these antimitotic factors is mostly unknown. Chalones, polyamines, or other nonspecific antiproliferative factors could be considered as the candidates for this role (27, 29). The concentration of these antimitotic factors probably increases with the size of the primary tumor mass. Thus, in the late stage of tumor growth, there is greater suppression of the proliferation of reinoculated tumor cells. The antiproliferative factors produced or induced by the primary tumor may inhibit the growth of metastatic cells that can be considered to be a spontaneous second tumor graft; hence, the accelerated growth of metastasis is manifested following removal of the primary tumor (13, 14, 27). It is possible that production of antiproliferative substances and the development of metabolic and hormone disorders in tumor-bearing animals could be responsible for suppression of the growth of reinoculated tumor cells or spontaneous metastases.

Therefore, the resistance of tumor-bearing and tumor-excised mice to the challenge of the second tumor is mediated by different mechanisms. There is increasing evidence that the resistance of a tumor-bearing host to the second tumor graft is mostly a nonimmunological phenomenon. However, antitumor immune response evoked against immunogenic tumor may contribute to the protection of the growth of the second tumor graft in the tumor-bearing host. Since the resistance to the second tumor challenge in tumor-bearing mice may be due to nonimmunological mechanisms, this experimental model cannot be used for the assessment of the antitumor immune reactions of tumor-bearing mice.

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REFERENCES


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