ABSTRACT

The role of concomitant and sinecomitant antitumor resistance in the regulation of metastatic outgrowth was assessed using methylcholanthrene (MCA)-induced tumors in C3H/HeJ mice. Variants of neoplasms MCA-F, MCA-D, and MCA-2A were selected for proclivity for spontaneous lung metastasis and expression of parental tumor-specific transplantation antigens. The incidence of spontaneous lung metastases after resection of a s.c. tumor of clone 9-4, a highly metastatic variant of the MCA-F tumor, was determined by both the size and the duration of neoplastic disease. The coexistence of the primary local tumor retarded lung colonization both from spontaneous and after artificially induced metastases. Greater concomitant immunity leading to a reduced number of artificial metastases after i.v. challenge with clone 9-4 cells was evident in hosts bearing large (1.6 to 1.8 cm) compared to small (0.1 to 0.2 cm) burdens of the nonmetastatic MCA-F ($P < 0.005$). Furthermore, i.v. challenge of mice bearing antigenically different tumors revealed that the concomitant inhibition was antigen specific with small tumor burdens, but nonspecific and possibly more efficacious with large tumor burdens. Therefore, concomitant antitumor immunity consists of both specific, immune-mediated resistance and nonimmunological mechanisms. Specific concomitant immunity decreases inversely with the progression of the primary, while nonimmunological inhibition of metastasis increases during late stages of primary growth. Abrogation of the strong nonspecific concomitant inhibition by resection of the primary tumor may facilitate lung metastasis. On the other hand, significantly greater inhibition of metastases occurred after resection of 7- or 14-day neoplasms compared to larger tumors ($P < 0.001$ or 0.05). Sinecomitant inhibition is antigen specific, probably representing an extension of specific concomitant immunity. These results suggest that adjunctive immunotherapeutic protocols for surgically treated hosts should augment existent specific immunity and promote nonspecific resistance, in order to minimize metastatic outgrowth.

INTRODUCTION

This investigation determined the intensity and specificity of two forms of tumor resistance: concomitant immunity displayed by hosts bearing a progressive primary tumor growth against a second tumor challenge; and sinecomitant resistance displayed to a second tumor challenge after excision of the primary tumor (1–5). The presence of a rapidly progressive primary neoplasm inhibits distant tumor growth in many murine models of metastasis. Excision of a s.c. primary tumor accelerates metastatic tumor growth in the lungs of mice (2, 6–8), while reinoculation of the primary tumor cells retards metastatic outgrowth (9). Concomitant antitumor immunity is indeed effective in the lungs (10). Since metastatic lesions are akin to small tumor burdens, but nonspecific and possibly more efficacious with large tumor burdens. Therefore, concomitant antitumor immunity consists of both specific, immune-mediated resistance and nonimmunological mechanisms. Specific concomitant immunity decreases inversely with the progression of the primary, while nonimmunological inhibition of metastasis increases during late stages of primary growth. Abrogation of the strong nonspecific concomitant inhibition by resection of the primary tumor may facilitate lung metastasis. On the other hand, significantly greater inhibition of metastases occurred after resection of 7- or 14-day neoplasms compared to larger tumors ($P < 0.001$ or 0.05). Sinecomitant inhibition is antigen specific, probably representing an extension of specific concomitant immunity. These results suggest that adjunctive immunotherapeutic protocols for surgically treated hosts should augment existent specific immunity and promote nonspecific resistance, in order to minimize metastatic outgrowth.

MATERIALS AND METHODS

Animals and Tumors. Three non-cross-reactive sarcomas (MCA-F, MCA-D, and MCA-2A) were induced in female inbred C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME) by s.c. injection of 3-methylcholanthrene and maintained by serial s.c. propagation in 5- to 7-wk-old female C3H/HeJ mice, as previously described (12).

Selection of Sublines. Sublines were obtained from a sixth in vivo passage, generation of MCA-F, MCA-2A, and MCA-D by a modification of the method of MacPherson (16). For example, after six in vivo passages, the MCA-F tumor was cultured to confluency and dissociated by treatment with 0.05% trypsin solution. One-tenth ml of a cell suspension (10 cell/ml) was dispensed into each well in a 96-well, flat-bottomed microtest tray (Falcon No. 3040), and single cell isolates were cultured in vitro, when the cells had grown to confluency. A cell suspension (10 cell/ml) was dispensed into each well in a 96-well, flat-bottomed microtest tray (Falcon No. 3040), and single cell isolates were cultured in vitro, when the cells had grown to confluency. A cell suspension ($10^6$ viable cells in 0.2 ml of HBSS) was inoculated i.v. at a dose of $10^6$ viable cells in 0.2 ml of HBSS into C3H/HeJ mice. On Day 21 the hosts were sacrificed by ether inhalation, and pulmonary colonies were dissected from normal tissue and cultured in vitro. When the cells had grown to confluency, $10^6$ viable cells were injected i.v. into naive C3H/HeJ mice. After this cycle was repeated 4 times, a daughter line was designated clone-9-4 for MCA-F, and for the other tumors by similar methods, MCA-2A-L and MCA-D-L. All sublines shared TSTA with the parental tumor by cross-immunoprotection tests.

Spontaneous and Experimental Metastatic Model. The number of spontaneous lung colonies was enumerated 14 or 21 days after resection of neoplasms generated by injection of $10^6$ (usually $10^6$) clone-9-4 cells into the left hind footpad or left flank. For the artificial metastasis model, tumors resected from 3 to 28 days after footpad or flank injection of $2 \times 10^7$ nonmetastatic MCA-F tumor cells were immediately challenged by tail vein inoculation of $5 \times 10^7$ clone-9-4, MCA-2A-L, or MCA-D-L cells. Pulmonary colonies were microscopically enumerated in mice sacrificed at 14 days or 21 days after tumor excision by India ink black ink insufflation by the method of Wexler (18).

The significance of differences in the number of metastatic colonies between groups was determined using the Kruskal-Wallis test; probability values were estimated from the $Q$ statistics calculated for each group.

Assessment of Immunity. LATA assessed the kinetics of cellular immunity in tumor-bearing mice. Spleen cells (2 x $10^6$), harvested at serial stages from tumor-bearing mice and admixed with $10^6$ MCA-F tumor cells (effector:target ratio, 200:1) in 0.2 ml of HBSS, were
RESULTS

Spontaneous Lung Metastases of Clone-9-4 Initiated with Different Numbers of Cells. The number of clone-9-4 tumor cells in the initial neoplastic inoculum determined the extent of spontaneous lung colonies at 21 days following left hind limb amputation. Ten cells failed to produce any colonies; 10³ or 10⁴ cells, 0 to 7 colonies; and 10⁵, 10⁶, or 10⁷ cells, 10 (range, 2 to 27), 9 (1 to 33), or 19 (12 to 62) colonies, respectively. The last three groups displayed a significantly greater number of colonies than the hosts given injections of 10³ tumor cells (Table 1). The group receiving 10⁶ cells had a significantly higher incidence of lung colonies than those hosts receiving 10³ or 10⁴ cells (P < 0.001). Thus there appeared to be a threshold size of the primary tumor in order to achieve metastases.

Effects of Tumor-bearing Time upon Lung Colonization. Varying numbers of lung metastases appeared when the tumor was amputated 3, 5, 7, 14, or 21 days after inoculation of 10⁴ clone-9-4 cells (Table 2). While hosts resected at 3, 5, or 7 days showed few lung metastases, there was an appreciable incidence of lung metastasis after 14 (median, 7; range, 1 to 35) or 21 (28; 5 to 75) days. Therefore, the extent of lung metastases was influenced by primary tumor size and/or the duration of tumor-bearing period before amputation.

Influence of Primary Tumor upon the Growth of Spontaneous Lung Metastases. The effect of the presence of a primary neoplasm upon the number of lung metastases was determined by amputation at varying times after footpad inoculation of 10⁶ clone-9-4. While nonamputees displayed few lung colonies 14, 21, or 35 days after implantation of clone-9-4, primary tumor resection on day 14 or 21 induced significant lung colonization at Day 35, namely 14 (0 to 130) or 30 (5 to 122) colonies, respectively (Table 3). Thus resection of the primary neoplasm facilitates lung colonization, and the presence of the primary neoplasm inhibits lung colonization, even in mice bearing tumors at the late stage of 5 wk.

Specificity of Inhibition of Lung Metastases in Tumor-bearing Mice. The immunological specificity of the inhibition of lung colonization by primary tumor was investigated using an artificial i.v. metastasis model. Mice already bearing large (>1 cm) or small (<0.5 cm) s.c. tumors in the flank and mice inoculated s.c. with 10⁴ MCA-F, antigenically distinct 10⁶ MCA-D, or 5 × 10⁵ MCA-2A-L cells were challenged i.v. with 5 × 10⁴ MCA-F clone-9-4 or MCA-2A-L cells (Table 4). Hosts displaying 1.3-cm primary tumors (Group 2) showed decreased numbers of not only homotypic MCA-F clone-9-4 [Table 4; 1 (0 to 4); P < 0.001], but also antigenically distinct MCA-2A-L [Table 4; 29 (12 to 57); P < 0.001] pulmonary colonies. Hosts bearing small primary tumors (Groups 3 and 4) showed specific inhibition of MCA clone-9-4 lung colonies (4 or 14; P < 0.001), but not of MCA-2A-L colonies (120 or 138; not significant). Mice given injections of 10⁶ MCA-D cells (Table 4) or MCA-F cells (Table 4) at the time of i.v. challenge did not inhibit lung colonization by either clone-9-4 or MCA-2A-L. On the other hand, mice bearing large MCA-D tumors did inhibit lung colonization by the antigenically distinct MCA-F clone-9-4 cells (P < 0.001). Thus hosts bearing a small tumor burden display specific concomitant immunity, while animals carrying a large tumor burden display nonspecific resistance to metastasis.

Specificity of Inhibition of Lung Metastases After Tumor Resection (Sinecomitant Immunity). Groups of mice either bearing or resected of tumors were challenged i.v. with 5 × 10⁴ clone-9-4 homotypic, MCA-2A-L, or MCA-D-L heterotypic cells. Mice bearing large 14-day MCA-F tumors nonspecifically retarded lung colonization by not only MCA-F clone-9-4, but also MCA-2A-L or MCA-D-L (P < 0.001 to 0.005). Hosts resected of primary MCA-F neoplasms showed specific inhibition of clone-9-4, but not MCA-2A-L or MCA-D-L lung metastases (Table 5). Thus tumor excision abrogates nonspecific resistance, thereby enabling measurement of specific antitumor immunity.

Influence of Tumor Size upon Artificial Lung Metastases in Tumor-bearing Mice. Since inhibition of metastatic growth by the primary tumor may be due to factors which affect tumor cells after they enter the vascular compartment, the influence of tumor size on metastasis was investigated. The effect of tumor size on the growth of both homotypic and heterotypic lung colonies was assessed in mice receiving 10⁶ clone-9-4 cells (Table 2) and 10⁴ MCA-D cells (Table 3). While nonamputees displayed few lung colonies 14, 21, or 35 days after implantation of clone-9-4, primary tumor resection on day 14 or 21 induced significant lung colonization at Day 35, namely 14 (0 to 130) or 30 (5 to 122) colonies, respectively (Table 3). Thus resection of the primary neoplasm facilitates lung colonization, and the presence of the primary neoplasm inhibits lung colonization, even in mice bearing tumors at the late stage of 5 wk.

Table 1 Influence of the size of the original clone-9-4 tumor inoculum on the incidence of spontaneous lung metastases

<table>
<thead>
<tr>
<th>No. of inoculated cells</th>
<th>Tumor wt at inoculation</th>
<th>Lung colonization incidence</th>
<th>Lung colonization</th>
<th>Median</th>
<th>Range</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10³</td>
<td>0</td>
<td>0/10</td>
<td>0</td>
<td>0</td>
<td>NS‡</td>
<td></td>
</tr>
<tr>
<td>10⁴</td>
<td>0</td>
<td>6/10</td>
<td>2</td>
<td>0–7</td>
<td>NS‡</td>
<td></td>
</tr>
<tr>
<td>10⁵</td>
<td>0</td>
<td>7/10</td>
<td>2</td>
<td>0–7</td>
<td>NS‡</td>
<td></td>
</tr>
<tr>
<td>10⁶</td>
<td>0.1</td>
<td>10/10</td>
<td>10</td>
<td>2–27</td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>10⁷</td>
<td>0</td>
<td>10/10</td>
<td>9</td>
<td>1–33</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>10⁸</td>
<td>0.6</td>
<td>10/10</td>
<td>19</td>
<td>12–62</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Footpad inoculation of clone-9-4 cells in 0.05 ml of HBSS.
‡ Mean tumor weight with each tumor weight calculated with the difference between the weight of the tumor-bearing leg minus the weight of control normal leg from normal mice.
‡‡ Statistical differences determined by the Kruskal-Wallis test comparing the incidence of lung colonies in each group to the 10³ group.
§ NS, not significant.

Table 2 Effect of tumor-bearing time upon the extent of lung colonization

<table>
<thead>
<tr>
<th>Resection day</th>
<th>Resected tumor wt (g)</th>
<th>Lung colony incidence</th>
<th>Lung colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.03</td>
<td>5/10</td>
<td>0–8</td>
</tr>
<tr>
<td>5</td>
<td>0.03</td>
<td>6/10</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>0.13</td>
<td>6/10</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>1.00</td>
<td>1.43</td>
<td>7</td>
</tr>
<tr>
<td>21</td>
<td>3.76</td>
<td>2.05</td>
<td>28</td>
</tr>
</tbody>
</table>

* Day of tumor-bearing leg amputation.
‡‡ Mean tumor weight calculated as described in Table 1.
‡‡‡ Statistical differences determined by the Kruskal-Wallis test; incidence of lung colonies in the Day 7 resection group was compared with other groups.
§ NS, not significant.

Table 3 Influence of the primary tumor upon the growth of spontaneous lung metastases

<table>
<thead>
<tr>
<th>Group</th>
<th>Primary resection</th>
<th>Day of sacrifice</th>
<th>n</th>
<th>No. of lung colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10³</td>
<td>14</td>
<td>12</td>
<td>NS‡‡</td>
</tr>
<tr>
<td>2</td>
<td>10⁴</td>
<td>21</td>
<td>10</td>
<td>0–1</td>
</tr>
<tr>
<td>3</td>
<td>Sham Day 14</td>
<td>35</td>
<td>12</td>
<td>1–8</td>
</tr>
<tr>
<td>4</td>
<td>Day 14</td>
<td>35</td>
<td>16</td>
<td>14–130</td>
</tr>
<tr>
<td>5</td>
<td>Day 21</td>
<td>35</td>
<td>15</td>
<td>30–122</td>
</tr>
</tbody>
</table>

* Statistical difference determined by the Kruskal-Wallis test; incidence of lung colonies in Group 3 was compared with other groups.
‡‡ NS, not significant.

IMMUNITY TO POSTSURGICAL METASTASIS
of primary tumor size upon lung colonization was tested in an artificial metastasis model in which mice bearing 0.1- to 0.2-cm or 1.6- to 1.8-cm nonmetastatic MCA-F tumors were given injections i.v. of 5 x 10^4 clone-9-4 cells. To avert death from local tumor burden, the primary, nonmetastatic MCA-F neo
tumor was excised 7 days after i.v. injection of the metastatic
plasm was excised 7 days after i.v. injection of the metastatic
human tumor, sham-operated

Influence of Tumor Size upon Artificial Metastases in Tumor-
excised Mice. The relationship of tumor progression to resist-
ance toward artificial lung metastases was tested by allowing an inoculum of 2 x 10^3 MCA-F tumor cells to grow prior to curative excision (Table 7). The number of lung colonies in
hosts after removal of a 3-day tumor (median, 23) was almost
the same as normal controls (median, 24). However, there was significant retardation of lung colonization at 7 days [2 (0 to 11); P < 0.001] or 14 days [10 (3 to 55); P < 0.05]. However, resection of 28-day tumors failed to induce inhibition. Spleen
cells from mice bearing MCA-F tumors for 3, 7, 14, or 28 days
were given injections s.c. of 2 x 10^5 MCA-F cells into the flank on Day 0. Each set of three groups was i.v. challenged with 5 x 10^4 of either clone-9-4, MCA-2A-L, or MCA-D-L cells. All mice were sacrificed on Day 14 to enumerate lung colonies.

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were tested for tumor neutralization in local adoptive transfer assays (Table 8). Spleen cells from hosts bearing neoplasms for 7 or 14 but not 28 days inhibited MCA-F tumor outgrowth significantly ($P < 0.05$). Therefore inhibition of experimental lung metastases at different stages of tumor development after excision paralleled the level of cellular immunity assessed in local adoptive transfer assays.

**DISCUSSION**

In the methylcholanthrene-induced murine fibrosarcoma model, the presence of a primary neoplasm retarded, but its resection facilitated, lung colonization. The incidence of postsurgical spontaneous metastases depends upon the stage of the tumor progression, namely both tumor size (Table 1) and duration of growth (Table 2). Presumably these factors affect both the number of cells which intravasate during tumor growth and the induction of antitumor immunity in tumor-bearing or tumor-rectected hosts. Although seeding of tumor cells is the lungs probably occurs prior to resection of the primary tumor, outgrowth is inhibited by concomitant immunity until the primary tumor is resected. Analysis of both concomitant and sinecomitant immunity revealed that the former may be specific or nonspecific, while the latter is TSTA specific. Furthermore, suppression of metastatic outgrowth by large s.c. growing primaries may be, in part, due to nonimmunological mechanisms. Nonimmunological inhibition of metastatic seedings may be due to single or to combination effects of growth factors and their respective receptors. As reviewed by Goustin et al. (19) growth factors may function between and among cell populations in various arrays, e.g., autocrine, paracrine. It may be that primary tumor growth regulates distal seeding by altering (a) tissue growth factor concentration, (b) presentation of growth factor receptors, or (c) production of biologically inactive competitors. At present we have no direct evidence to support any single hypothesis. Furthermore, the production of a single metastasis inhibition factor by the primary tumor remains a plausible, yet undocumented, mechanism.

To observe the effects of the presence or the removal of a primary tumor on metastases, the artificial model was studied. Using the artificial metastasis model, the number of lung colonies following i.v. injection was greater in mice bearing small tumors than in animals with large tumor burdens (Table 6). Similar results were obtained in concomitant inhibition of s.c. inoculated Meth A in BALB/c mice (3), 3LL in BALB/c × DBA/2 F, mice (3), or EL4 lymphoma in C57BL/6J mice (10), and in pulmonary metastases following i.v. inoculation of methylcholanthrene-induced syngeneic murine sarcoma in C3Hf/Bu mice (20). Thus, concomitant inhibition is stronger in hosts bearing large tumor burdens than small tumor ones. Investigation of the components of the immune response was performed using isolated spleen cells. This population was chosen because it is well characterized and conveniently obtained as a representative population. However, the degree of inhibition of metastases is not necessarily reflected in the tumor-neutralizing activity of spleen cells from tumor-bearing donors and may be better reflected in the lymphoid populations of the lung. The inhibition of lung colonization in whole-body irradiated mice by spleen cells transferred from tumor-bearing mice was optimal 12 days after tumor inoculation (19). Further, the comparison of concomitant suppression of metastasis with kinetics of cellular immunity (LATA) during tumor progression in mice (Table 8) showed that, when the primary tumor was large enough to nonspecifically suppress metastasis, the specific cellular immunity as determined by LATA was weak. In contrast, inhibition of lung colonization after excision of a relatively small tumor (7- or 14-day tumor) was much stronger than after excision of a large tumor (Table 7). North and Bursuker (4) reported that the concomitant immunity was generated 6 days after 10⁶ Meth A challenge, peaked on Day 9, and then rapidly decayed. They also showed that the postexcision immunity after the removal of primary tumor on Day 16 was down-regulated by the activity of T-suppressor populations in tumor-bearing hosts (5). The inhibition of lung colonization presented herein paralleled the cellular immunity measured by LATA at the time of tumor excision (Table 8). Further, the inhibition after tumor resection was antigen specific (Table 5). Therefore after resection of a small tumor resection, sinecomitant immunity does not inhibit colonization by an antigenically different variant.

Antigenic differences between the parental tumor and its metastases have been reported in several systems (21–23). Possibly the exquisite specificity of sinecomitant immunity is one of the reasons that metastatic facilitation may occur even after removal of a relatively small tumor. In mice bearing large tumors, concomitant inhibition of lung colonization was nonspecific (Table 4). Gorelik (3) also showed that suppression of reinoculated radiolabeled M109 tumor cells (2 x 10⁶) into BALB/c or BALb/c nude mice bearing a tumor greater than 2 cm was the same in both strains and survived to the same extent at the local site of inoculation in tumor-bearing and non-tumor-bearing control mice. The growth of reinoculated 3LL, B16, EL4, or T-10 cells was equally inhibited in thymectomized, irradiated, and bone marrow reconstituted C57BL mice and nude mice as well as immunologically competent mice bearing tumors more than 1.5 cm (2). Therefore all primary tumors when large enough inhibit not only immunogenic, but also low or nonimmunogenic tumors whether in immunocompetent or immunodeficient hosts. These results suggest that, in addition to immunity, other nonimmunological host factors may regulate the rate of tumor cell proliferation at specific tissue sites in hosts bearing large tumor burdens. The induction of nonspecific concomitant tumor inhibition has not been well understood. Niederkorn and Streilein (24) indicated that immunologically privileged, intracamerally inoculated P815 mastocytoma cells induced specific concomitant immunity which was T-cell dependent and radiosensitive, but because the intraocular tumor is small, the mice display specific concomitant immunity.

 Serum factors in mice bearing methylcholanthrene-induced sarcoma did not influence the number of pulmonary metastases (20, 25). Unfortunately, estimation of the serum content of TSTA is not feasible because of the lack of specific antibodies. Gorelik (3) showed that some concomitant suppression of metastasis was not due to T-cells, natural killer cells, or macro-

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**Table 8. Kinetics of cellular immunity in MCA-F-bearing mice**

<table>
<thead>
<tr>
<th>Tumor-bearing day</th>
<th>n</th>
<th>Tumor size (mm)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10</td>
<td>11.0 ± 0.27</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>12.8 ± 0.76</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>8.0 ± 1.33</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>8.3 ± 1.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>28</td>
<td>10</td>
<td>11.6 ± 1.49</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Statistical differences were determined by Student's t test by comparison with reactions containing normal spleen cells.

$^*$ Mean ± SE.

NS, not significant.
phages, but probably to nonimmunological mechanisms in mice bearing nonimmunogenic tumors and to antitumor immune reactions with additional nonimmunological mechanisms in mice bearing immunogenic tumors. The experiments using the methylcholanthrene-induced sarcoma described herein suggest that there are two types of concomitant inhibition. The one is specific cellular immunity, which is dominant in small tumor-bearing mice and decreases with tumor growth. The other is nonspecific inhibition, possibly nonimmunological mechanisms, which prevails in animals with large tumor burdens and increases with tumor growth. The nonspecific inhibition is strong, but disappears with tumor excision. This may be a contributory factor to the metastatic facilitation that follows resection of large tumors. The sinecomitant immunity, which is presumably the continuation of the specific cellular concomitant immunity, depends upon the tumor stage at resection, and it does not afford resistance to antigenically different metastatic foci (Table 5). Since postsurgical therapy with immunospecific reagents probably augments specific immunity, control of postsurgical metastasis may require additional activation of nonspecific immune and nonimmunological factors to control proliferation of antigenically distinct metastatic foci.

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Effects of Concomitant and Sinecomitant Immunity on Postsurgical Metastasis in Mice

Shinhachiro Nomi, Kazuyo Naito, Barry D. Kahan, et al.


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