Distant Metastasis from Subcutaneously Grown E0771 Medullary Breast Adenocarcinoma

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Abstract. Background: Breast cancer treatments are most effective when initiated early, with very poor efficacy against metastatic disease. In seeking a readily metastasizing mouse breast cancer model to facilitate the search for effective therapies, E0771 medullary adenocarcinomas implanted subcutaneously in syngeneic C57BL/6 mice were studied. Materials and Methods: Standard pathological, histological and immunological methodologies were used. Results: The aggressive estrogen receptor-positive tumor invaded locally into the peritoneal cavity in 56% of mice, as well as metastasizing to the lungs in 52% of mice. The metastasis was a spontaneous event and immunosuppression was seen (e.g. generation of lymphokine activated killer cells and allogeneic cytotoxic T lymphocytes cytolytic activities ex vivo were suppressed). Other pathological events noted as the tumor progressed were: bloody ascites (56%) and shock (72%), both attributed to local (peritoneal) tumor invasion. Conclusion: The E0771 metastatic breast cancer model, which mimics the human disease, should be useful in testing new treatments against this disease and/or in examining the metastatic process.

Each year in the United States over 210,000 new cases of breast cancer are diagnosed, resulting in almost 40,000 deaths (1). Seventy to eighty percent of breast cancers are diagnosed at an invasive stage (2-4). Breast cancer invasion can occur to the skin, chest wall, contra lateral breast, lymph nodes, bone, lungs, liver and brain (4). At 5 years post diagnosis, stage I breast cancer is associated with 87% survival while only 13% of those with metastasis at diagnosis survive (5). The rate of death from breast cancer has stayed relatively constant since before 1940 (6).

The inability to effectively treat metastatic disease has contributed to the stagnant rate of survival (7, 8). Most cancer model systems used to develop new treatments utilized tumors which were grown subcutaneously and rarely metastasize (8-11). Artificial models based on intravenously injected cancer cells have been developed to try to mimic metastasis in order to test the effectiveness of new cancer treatments (12, 13). This approach results mainly in tumors of the lung since the lung contains the first capillary bed after injection where the cell aggregates become lodged (14). Therefore, these artificial models are devoid of most of the rate-limiting steps of metastasis (e.g. detachment and invasion of cancer cells through normal tissue into blood vessels).

Many human cancers are also able to suppress the activity of the immune system (15, 16). The immunosuppressive phenomena are generally associated with a poor prognosis and offer a challenge to immune-based therapies (17-19). A model system in which immunosuppressive tumors metastasize distantly would offer an excellent opportunity to closely model human disease and would be useful for developing effective new drugs or for basic studies on the metastatic process. E0771 medullary breast adenocarcinoma cells, originally isolated from a spontaneous tumor, are syngeneic to C57BL/6 mice and readily grow subcutaneously (20, 21). The characterization of the subcutaneously implanted E0771 tumor cells in C57BL/6 mice, focusing on immunosuppression and metastasis, is reported herein.

Materials and Methods

Cell lines. E0771 medullary breast adenocarcinoma cells were obtained from Dr. F. M. Sirotnak at Memorial Sloan-Kettering Cancer Center, New York, NY, USA. E0771 cells were originally isolated from a spontaneous cancer in C57BL/6 mice (20, 21). The E0771 cells were maintained as a monolayer in RPMI 1640 supplemented with 10% calf serum with iron (Gibco/BRL, Grand Island, NY, USA) and 10 mM HEPES buffer at 37°C, 100% humidity and 5% CO2. They are estrogen receptor-positive. The tumor cell lines (EL4 lymphoma, YAC-1 lymphoma and P815 mastocytoma) used in the immuno-cytolytic studies were maintained as previously reported (22).
Mice. Female C57BL/6 mice were obtained through the National Cancer Institute grantee program, Frederick, MD, USA. Mice were kept on corn cob bedding in sterilized filter top cages with controlled humidity and 12-hour day/night cycles at 72 °C. The mice were fed sterilized LM-485 rodent chow (Harlan Teklad, Madison, WI, USA) and water was provided ad libitum. Experiments were started when the mice were 8 to 12 weeks old. Individual mice were identified by 1 of 5 ear hole punch patterns. All aspects of animal experimentation at AAALAC accredited RPCI has had prior approval of the Animal Care and Use Committee and are in compliance with all state and federal regulations.

Cancer cell injections. E0771 cells were harvested for injection into mice by trypsin digestion (1 ml of 0.05% trypsin-EDTA) for 5 minutes. The cells were washed with Hank’s Balanced Salt Solution, counted, diluted in this salt solution and subcutaneously (s.c.) injected near the fat pad of the fourth mammary gland in the lower abdomen at 2.5x10^5 cells/200ml/mouse.

Tumor measurement. Using dial calipers, the tumors were measured in two perpendicular dimensions parallel with the surface of the animal; the first dimension being the tumor’s longest and designated its length. The size of the tumor is expressed as: length(mm) x width(mm) = tumor size(mm^2).

Necropsy/histological analysis. The animals were euthanized by CO2 asphyxiation when their tumors had reached 20 mm in the longest dimension. Animals whose tumors reached this size are considered to have a lethal disease (i.e. survival free of tumor was no longer possible). Their abdominal and thoracic cavities were opened and examined. Photographs at the time of necropsy were taken of tissues displaying representative pathologies. Selected tissues were taken for histological examination depending on gross findings. Tissues of interest were placed in formalin and given to the Roswell Park Cancer Institute Preclinical Histology Facility for processing and hematoxylin/eosin staining. Stained tissues were examined.

Immune cell cytolytic activity assay. The effects of E0771 tumors on immune cell cytolytic activities were measured ex vivo using published procedures (22). Briefly, using splenocytes (1x10^6 cells/100 µl/well of a round bottom 96-well culture plate), the activity of either lymphokine activated killer (LAK) cells or cytotoxic T lymphocytes (CTL) were determined following 5-day stimulation cultures. For LAK cytolytic activity, splenocytes were stimulated in a 5-day culture with IL-2 (DuPont de Nemours & Co., Glenolden, PA, USA), added at 5.2x10^4 IU IL-2/100 ml/well. For CTL cytolytic activity, stimulator cells (same cell type as target cells) were lethally X-irradiated and added to the 96-well plates in 100 ml to give splenocyte (effector) to stimulator cell ratios of 1:0, 5:1, 100:1. Stimulated effector splenocytes were serially diluted (2-fold) 4 times. Cultured target cells (YAC-1, P815, EL4 and E0771 for LAK activity and E0771 or P815 for CTL activity) were harvested, labeled with Chromium-51 (51Cr, PerkinElmer Life Sciences Inc., Boston, MA, USA) and water was provided

\[ \text{percent specific release} = \frac{\text{avg count experimental} - \text{avg count spontaneous release}}{\text{avg count maximum release} - \text{avg count spontaneous release}} \times 100 \]

Data presentation and statistical analysis. The data were analyzed by simple descriptive statistics of mean and standard deviation using Microsoft Excel 2000 software from Microsoft, Seattle, WA, USA. Further statistical analysis was not performed because of the obvious significance.

Results

E0771 tumor growth rate. The growth of primary E0771 tumors was examined. The parameters that were evaluated included: the percentage of the mice injected with E0771 cells in which the tumor grew, the amount of time it took for the primary tumor to double in size and the time to euthanasia based on tumor burden. These characteristics were evaluated in 3 independent experiments involving a total of 62 mice.

The in vivo growth characteristics of the E0771 tumor in the C57BL/6 mouse were examined following the s.c. injection of cultured E0771 cells (2.5x10^5 cells/mouse). The tumor size was measured every 2 to 3 days. The experimental end-point was tumor size. Mice were considered to have lethal disease and were euthanized when their tumor reached or just exceeded 20 mm in the greatest dimension. Any mice in which a tumor did not grow after the initial injection of E0771 cells were re-injected with E0771 cells (2.5x10^6 cells/mouse) at a point in time after all of the other mice in that experiment had been euthanized. Animals receiving a second E0771 cell injection were monitored for tumor growth as was done previously. The data pooled from the 3 experiments are shown in Figure 1. The results show that, after the first injection of E0771 cells, tumors grew in 97% (i.e. all but 2) of the mice and that all of these mice had been euthanized due to high tumor burden by 50 days post tumor injection. The 2 mice out of 62 which did not initially grow a tumor, were re-injected 62 days after the first E0771 cell injection and tumors grew in both mice.

By evaluation of the rate of growth over the linear portion of the growth curve, the time for the E0771 tumors to double in size was determined to be 7 days. Injection of cultured E0771 cells into C57BL/6 mice resulted in tumor growing successfully in all of the mice.

E0771 tumor induced immunosuppression. Many tumors are able to suppress immune responses as a survival mechanism
and the ability to do so would be expected to impact the effectiveness of an immune-mediated therapy. The ability of E0771 tumors to suppress immune responses were assessed by comparing LAK and CTL cytolytic activities of splenocytes from naive mice with those from E0771 tumor-bearing mice (Figure 2). Spleen (effector) cells from E0771 tumor-bearing mice (33 days after E0771 cell injection) and naive mice were stimulated by incubation with IL-2 (LAK generation) or with lethally X-irradiated stimulator cells [allogeneic (P815 cells) or syngeneic (E0771 cells) CTL generation] for 5 days. Cytolytic activity was measured by combining those stimulated spleen (effector) cells with radio-labeled (51Cr) target cells (Figure 2). For analysis of LAK cytolytic activity, a panel of tumor cells was used as targets. The same tumor cells used in the generation of CTL cultures were used as targets in the CTL cytolytic activity assay. The 51Cr released from lysed target cells into the supernatant was measured to assess the amount of cytolytic activity. The activity is expressed as the average percent specific 51Cr release of 6 replicate wells. Compared with splenocytes from naive mice, those from E0771 tumor-bearing mice had significantly lower LAK cytolytic activities against YAC-1 (0% tumor vs. 17% no tumor), E0771 (3% tumor vs. 19% no tumor) and P815 (0% tumor vs. 8% no tumor) cells. The LAK cytolytic activity against EL4 cells was too low to compare. Splenocytes from tumor-bearing mice had significantly lower CTL cytolytic activity against P815 (1%) than those from naive mice (17%). As would be expected with a syngeneic tumor, splenocytes from neither naive nor tumor-bearing mice were able to generate a CTL response against syngeneic E0771 tumors and, thus, suppression could not be assessed.

Pathological evaluations. Progressing E0771 tumors were evaluated by non-invasive and necropsy (gross and histological) observations made in 2 independent experiments. These experiments examined primary and secondary tumor development and pathologies. The frequency of pathologies was determined from a total of 25 mice. The mice were observed every 1 to 3 days and were necropsied when their primary tumor reached 20 mm in the longest dimension. Photographs of representative gross pathologies were taken and the tissues were processed and stained with hematoxylin/eosin for histological examination.

Primary E0771 tumors: At necropsy, the s.c. implanted primary E0771 tumors were found to be growing in
Figure 2. Comparison of LAK and CTL cytolytic activities generated with splenocytes from non-tumor-bearing mice and with those from E0771 tumor-bearing mice. Spleen (effector) cells from E0771 tumor-bearing mice (33 days after E0771 cell injection) and from naive mice were prepared by passage through stainless steel mesh and were plated (1x10^7 cells/ml) in 96-well plates. LAK cytolytic activity was measured after the effector cells had been stimulated with IL-2 (5.2x10^5 IU/ml) for 5 days [A]. CTL cytolytic activity was measured after the effector cells had been stimulated with lethally X-irradiated stimulator cells (P815 for allogeneic response [B], E0771 for syngeneic response [C]) at an effector to stimulator cell ratio of 50:1 for 5 days. Cytolytic activity was assessed by co-incubation with ⁵¹Cr-labeled target cells (YAC-1, E0771, P815 and EL4 for LAK response [A], P815 for allogeneic CTL response [B] and E0771 for syngeneic CTL response [C]) at effector to target cell (E:T) ratios of 100:1, 50:1, 25:1 and 12.5:1 for 5 h. The resulting released ⁵¹Cr was measured and expressed as percent specific ⁵¹Cr release normalized to untreated target cells (spontaneous release = 0% specific release) and detergent-lysed target cells (maximum release = 100% specific release). The average and standard deviations of activity from 6 replicate wells for each assay condition are shown for each target cell tested. The key to the data bars giving the source of effector cells first followed by target used is as follows:
association with (invasion into) either the dermal layer, the peritoneal/abdominal muscle wall or both (Figure 3A). By retracting the skin away from the peritoneum, the difference in the 2 tumor locations could be visualized. Primary tumor growth within the peritoneal wall, with or without dermal involvement, was seen in 76% of mice with 8% of primary tumors associated with the dermal layer without any involvement with the peritoneal wall. Many primary tumors growing in the peritoneal wall protruded into the peritoneal cavity (Figure 3B). Primary E0771 tumor tissue was taken during necropsy for histological examination. At low magnification, the border of the primary tumor, consisting of undifferentiated cells, can be seen invading into the dermis and a necrotic center (area devoid of nuclei and full of debris) can be seen within the tumor (Figure 3C). The edge of a necrotic center is shown at high magnification (Figure 3D) and leukocyte infiltration is evident, composed mainly of neutrophils and some lymphocytes. The number of necrotic foci, the size of the foci as well as the extent of leukocyte infiltration varied among primary tumors.

Figure 3. Gross and histological examination of primary E0771 tumors. A primary E0771 tumor is shown before the peritoneum was opened (A) and after it was opened (B), showing a cross-section of the peritoneal wall and tumor protruding into the peritoneal cavity (large arrow). A low magnification (100x) hematoxylin/eosin-stained tissue section (C) shows the border of a tumor (area of tumor designated T in the dermal layer (area of normal tissue designated N) and a necrotic center (arrow). A higher magnification (200x) of a hematoxylin/eosin-stained section from an E0771 tumor (D) shows leukocyte infiltration, consisting primarily of neutrophils (circled) at the edge of a necrotic center (arrow).
Ulceration of the primary tumor was observed in 12% of mice. Interestingly, no behavioral changes were noted in mice with ulcerated primary tumors.

Secondary E0771 tumors of the peritoneum: During necropsy, secondary tumors were found within the organs of the peritoneal cavity in 56% of mice. The sites for secondary tumor colonization were in the intestinal mesentery, pancreas, diaphragm and at sites on the peritoneal wall distant from the primary tumor (Figure 4A-H). Although, the secondary tumor was often restricted to a single site, it was seen that secondary tumors could occur at different sites within the peritoneum of a single mouse. Based on morphological criteria, these secondary tumors were concluded to have arisen from the primary E0771 tumor. The relative frequencies of the occurrences of secondary tumors at the various sites were as follows: intestinal mesentery (48%), pancreas (44%), diaphragm (32%) and peritoneal wall sites distant from the primary tumor (12%). Secondary tumor growth in the mesentery ranged from individual pink foci (not shown) to confluent multi-focal masses with gross hemorrhage (Figure 4A). Histologically the tumors found in the mesentery had replaced the normal tissue and were hemorrhagic (Figure 4B). Secondary tumors in the pancreas were usually confluent, multi-focal masses found under the stomach and spleen (Figure 4C). Histologically, progressive replacement of normal pancreatic tissue was usually seen, often with complete replacement of several lobes (Figure 4D). Secondary tumors in the diaphragm were usually of a single thick opaque focus taking up less than 25% of the diaphragm (Figure 4E). Histologically the tumors adhered to and replaced the muscle of the diaphragm (Figure 4F). Secondary tumors in the peritoneal wall were thick, bumpy and opaque, occasionally with hemorrhage (Figure 4G). Histologically, tumor infiltration into muscular tissue could be seen with replacement of normal tissue (Figure 4H).

Secondary E0771 tumors of the lung: In addition to secondary tumors found in the peritoneum, more distant secondary tumors were found in the lungs (Figure 5). Secondary tumors in the lungs were seen in 52% of the mice. Grossly, 2 variants of these lesions were seen, either as a clear raised spot (~2/3 of the cases) and/or a red raised spot (~1/3 of the cases). The severity of lung tumors varied from a single small lesion to large multiple lesions on different lobes of the lung which often included both variants. Histologically the lesions were seen to have replaced normal lung tissue and included not only tumors observed at necropsy, but also multiple micro-metastatic tumors throughout the lung tissue. These secondary tumors of the lung were found to have the same morphology as primary E0771 tumors. There were
leukocytes, composed mainly of neutrophils with lymphocytes also present, surrounding the tumors in some cases. The difference between the clear and red lesions was defined histologically as the presence of hemorrhage, since large numbers of red blood cells were observed outside the confines of the vasculature. The percentage of lung metastases reported above was a conservative estimate of the true frequency of tumors as it was based on gross observations and, therefore, failed to include mice with micro-metastases without gross signs of lung tumors.

Ascites: Prior to necropsy, ascites, presenting as an abdominal swelling to a size larger than the rib cage (Figure 6A), were observed in 44% of mice. The ascites were determined at necropsy to be accompanied by an accumulation of blood in the peritoneum (Figure 6C). Mild to moderate amounts of ascites did not significantly alter the mouse behavior. In very rare instances of severe ascites, it was noted to cause labored breathing and slowed movement. Additional mice (12%), that had not been observed to have ascites prior to necropsy, were found to

Figure 5. Gross and histological examination of secondary E0771 tumors of the lung. Secondary E0771 tumors are seen grossly (arrows) in the lung (A) and histologically in a hematoxylin/eosin-stained tissue section (B) as a micro-metastatic tumor, not detectable grossly (arrow). The tumors in the lung were seen grossly as either clear (not shown) or red (A) lesions. Upon examination of hematoxylin/eosin-stained tissue sections, clear lesions were seen as non-hemorrhagic E0771 tumors (C), while red lesions were tumors that contained significant amounts of hemorrhage (D), as indicated by the presence of extra-vascular red blood cells (circled). Magnification: 100x panels B, C and D.
have bloody ascites at necropsy. Therefore, a total of 56% of the mice were demonstrated to have developed ascites.

**Liver necrosis:** Lesions in livers were seen by gross observation during necropsy in 20% of mice. These lesions were seen as yellow or brown spots on the liver (Figure 6F), frequently accompanied by large gall bladders (not shown). Histologically, these lesions revealed sites of coagulative necrosis with leukocyte infiltration, consisting mostly of neutrophils, in or around the necrotic sites (Figure 6G). Interestingly, E0771 tumor cells were never observed in livers, with or without lesions.

**Observed shock.** Shock was seen in 72% of mice with late-stage disease. Shock was designated by the appearance of pale paws and digits (change from pink tone to gray tone, Figure 6E), while ruffled hair, prostration and dyspnea with shallow breathing were often observed. Occasionally, shock was accompanied by possible hypothermia, based on the subjective evaluation of the mouse’s external body temperature. These criteria are the ones others have used to assess the incidence of shock (23). Shock was seen mostly as a temporary condition; only 8% of mice suffered lethal shock.

**Relationship between primary tumors and pathologies.** It was suspected that the secondary tumors seen in the peritoneum were a result of invasion of primary tumor through the peritoneal wall, while the secondary tumors in the lung were distant metastases. This hypothesis was examined using data from a total of 69 mice, the premise being that distant metastases should arise equally from primary tumors without peritoneal wall involvement and from those with peritoneal

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Figure 6. Observed pathologies. Ascites was observed non-invasively in a tumor-bearing mouse as a swollen abdomen (A), compared to the abdomen of a naive mouse (B). Bloody ascites was identified through the peritoneal wall in a mouse with pools of blood (arrows) between the abdominal organs (C). Shock was observed non-invasively in a tumor-bearing mouse as pale-colored paw and digits (D), compared to the paw and digits of a naive mouse (E). Liver lesions were seen grossly (arrow) in a tumor-bearing mouse as yellowish-brown spots (F). Liver lesions were identified by examination (100x) of a hematoxylin/eosin-stained tissue section as coagulative necrosis (a nuclear area which retains normal structure, designated Nec) with leukocyte infiltration (G).
wall involvement, while secondary tumors of the peritoneum should arise primarily from the latter. The distribution of mice with specific pathologies, that had either primary tumors with peritoneal wall involvement or with tumors confined to the dermis, was determined. To allow a direct comparison, the total numbers of mice with each primary tumor localization type need to be equal. Since fewer mice had primary tumors confined to the dermis than those involving the peritoneal wall, a normalization factor was generated to equalize the number of mice with primary tumors from the 2 different locations. The total number of mice with intraperitoneal involvement of their primary tumor at the time of necropsy (54 mice) was divided by the total number of mice with dermally-confined primary tumors at the time of necropsy (15 mice) to calculate the normalization factor (54/15 = 3.6). The normalization factor was then multiplied by the total number of mice which had dermally-confined primary tumors and the number of such mice presenting with each pathology. Then, using these normalized numbers of mice, the percentage of mice with a specific pathology that occurred in mice with either primary tumors in their peritoneal wall or confined to the dermis were determined (Table 1). Of those mice with secondary tumors in the peritoneum, 92% had primary tumor involvement in the peritoneal wall and only 8% had dermally-confined primary tumors, suggesting that the secondary tumors found in the peritoneum arose from local invasion. Of those mice with secondary tumors in the lungs, 45% had primary tumor involvement in the peritoneal wall and 55% had dermally-confined primary tumors, consistent with secondary tumors arising in the lung as a result of metastasis. Over 97% of mice with either shock or observed ascites had primary tumor involvement in the peritoneal wall, suggesting the association between these pathologies and locally invasive tumors.

### Discussion

Tumor take occurred in 97% in of the mice following the initial injection of E0771 cells. The failure of E0771 cells to grow in 3% (2/62) of the mice was most likely a technical problem (e.g. a result of E0771 cells leaking out of the injection site), since tumors did grow in both mice after the second injection of E0771 cells. If the lack of E0771 tumor development following the initial injection had been a result of an anticancer immune response, then it would have been expected that immune memory against E0771 cells, induced subsequent to the initial E0771 cell injection, would have prevented tumor growth after the second injection. Additionally, the lack of an anti-E0771 immune response was supported by the observation that splenocytes from naive mice were unable to develop an ex vivo CTL response against E0771 cells. These results suggest that E0771 tumors are poorly immunogenic. Splenocytes from E0771 tumor-bearing mice did not respond in ex vivo allogeneic CTL and LAK cytolytic activity generation cultures, whereas splenocytes from naive mice did. These results suggest that progressively growing E0771 tumors are non-specifically immunosuppressive. Clinically, tumors which are not only poorly immunogenic, but also immunosuppressive are frequently associated with a poor prognosis (17-19). The immunosuppressive nature of the E0771 tumors grown in C57BL/6 mice makes this a challenging model in which to test immune-based therapies.

Histologically, primary E0771 tumors had recognizable borders and were composed of undifferentiated cells. Multiple necrotic centers surrounded by leukocytes were present. These histological findings were also seen in human medullary breast cancer (1).

Primary E0771 tumors grew either attached to the peritoneal wall, the dermis or both. Secondary tumors were seen at multiple sites in the peritoneal cavity and in the lungs and all had morphology similar to that of the primary tumor. Secondary tumors in the peritoneal cavity occurred almost exclusively (92%) in mice in which the primary tumors grew within the peritoneal wall, many of which protruded into the peritoneal cavity. The peritoneal wall serves as only a minor barrier to invasion and so secondary tumors in the peritoneal cavity were almost certainly caused

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**Table 1. Association between primary tumor invasion sites and specific pathologies suggests their etiology.**

<table>
<thead>
<tr>
<th>Site of local invasion</th>
<th>Lung tumors (% associated with observed site of invasion)</th>
<th>Peritoneal tumors</th>
<th>Ascites</th>
<th>Shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermal only</td>
<td>55c</td>
<td>8</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>Peritoneal wall ± dermal</td>
<td>45c</td>
<td>92</td>
<td>98.5</td>
<td>98</td>
</tr>
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aDuring 2 independent experiments, a total of 69 E0771 tumor-bearing mice were evaluated.

bMice were categorized by the site of the local invasion of their primary tumor [i.e. confined solely to the dermal layer (dermal only) or involving the intraperitoneal wall with or without the dermal layer (interperitoneal ± dermal)].

cThe total number of mice with primary tumor invasion confined to the dermis was normalized to that with intraperitoneal involvement. The adjusted numbers of mice with each pathology were then used to compare the percentage of mice with the given pathology between the 2 categories of mice with different primary tumor locations.

dThe suggested tumor-induced etiology of the pathologies are given.

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Naive mice cannot develop an ex vivo CTL response against E0771 cells. These results suggest that E0771 tumors are poorly immunogenic. Splenocytes from E0771 tumor-bearing mice did not respond in ex vivo allogeneic CTL and LAK cytolytic activity generation cultures, whereas splenocytes from naive mice did. These results suggest that progressively growing E0771 tumors are non-specifically immunosuppressive. Clinically, tumors which are not only poorly immunogenic, but also immunosuppressive are frequently associated with a poor prognosis (17-19). The immunosuppressive nature of the E0771 tumors grown in C57BL/6 mice makes this a challenging model in which to test immune-based therapies.
by local primary tumor invasion. Secondary tumors in the lungs were grossly observed in 52% of the mice and consisted of 2 forms. The 2 forms, red or clear, were differentiated by the presence of hemorrhage, possibly reflecting different stages of tumor progression. The reported frequency of secondary tumors in the lungs was based on gross observation, but lungs were also found to contain multiple micro-metastases. The estimation of the frequency of lung tumors based on gross observation is, therefore, a conservative estimate of the true frequency. Secondary tumors in the lung are thought to represent distant metastasis. Additionally, the lungs contain the first capillary bed that blood-borne cancer cells would reach and is a common site of distant metastasis in patients with breast cancer and many other types of cancers (1, 4). Finally, it was found that secondary tumors in the lung were equally likely to occur in mice with either intraperitoneally-involved primary tumors (45%) or those confined to the dermis (55%), indicating that lung metastasis was not dependent upon the local invasion of the peritoneal wall by the primary tumor. This is consistent with metastasis to the lungs through a systemic route (vascular or lymphatic). Other common sites of human breast cancer metastasis include bone, brain, liver and adrenal glands, but these sites were either not examined (bone and brain), or did not demonstrate the presence of tumors (liver and adrenal gland) in the E0771 model. Additional pathologies, which included bloody ascites, shock and necrosis of the liver, occurred in some mice. These pathologies rarely (8%) became lethal before the mice had to be euthanized because of a large primary tumor size. Interestingly, liver necrosis was seen in 20% of mice, but was not always fatal. Necrosis of the liver was seen in 20% of mice, but was asymptomatic and only detected during necropsy after euthanasia due to the primary tumor size. Interestingly, liver necrosis was only seen in tumor-bearing mice, even though E0771 cells were not detected histologically in the liver of these mice. The etiology of this sequela is unknown.

Human breast cancer often metastasizes to the lungs and can be highly invasive locally (4). The invasiveness of E0771 is unique among tumors grown subcutaneously (9-11), suggesting that E0771 tumors grown in the C57BL/6 mouse model represent human disease better than most other subcutaneously grown tumors. The immunosuppressive nature and invasiveness of E0771 tumors, which are similar to those seen in the human disease (15-17), suggest that this model system would provide a significant challenge to therapies, especially immunotherapies. Effective treatment against E0771 tumors would be consistent with the possibility that such a treatment would have clinical importance. In addition, this model would also be useful for basic studies of the metastatic process.

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References


