Specificity of Arrest, Survival, and Growth of Selected Metastatic Variant Cell Lines

Garth L. Nicolson, Kenneth W. Brunson, and Isaiah J. Fidler

Abstract

Animal tumor models for blood-borne metastasis have been developed by in vitro cloning or in vivo selection of malignant tumor cell populations to obtain organ-prefering variant cell lines with altered arrest, survival, invasion, and growth properties. Selection and some tumor cell characteristics of lung-, brain-, and ovary-colonizing metastatic B16 melanoma, liver-colonizing RAW117 lymphosarcoma, and lung-colonizing MSV3T3 vasoformative sarcoma variant lines will be discussed along with additional data, suggesting that tumor cells of varying malignant potential preexist in the unselected tumor population.

Introduction

The most life-threatening characteristics of malignant tumor cells are their abilities to detach from the primary tumor, invade into surrounding normal tissues, and form metastatic growths at distant sites (13, 22, 47). Metastatic colonies can be close to or distant from the primary site, and the latter may occur when tumor cells penetrate the lymphatics or circulatory system. Of particular interest are malignant cells that enter the bloodstream, because these can be transported to the major organs. However, tumor cell survival during blood-borne transit is not a common event, since the overwhelming majority of malignant cells expire in the circulation (15, 39).

The survival of malignant cells after they enter the circulation depends upon their abilities to successfully implant in the microcirculation, invade or extravasate the capillary endothelium, and establish a microenvironment for subsequent vascularization and growth (13, 22, 47). While in the blood, the distributions of viable malignant cells are not necessarily based on circulatory pathways or patterns of initial microcirculatory arrest (20, 42). Blood-borne tumor cells can be temporarily arrested in the first capillary bed encountered, but at this point the cells may: (a) die; (b) survive and grow at the site of implantation (c) deform and recirculate to be arrested at other sites; or (d) invade and survive at extravascular sites. In a variety of experimental blood-borne tumor systems, the distributions of gross metastases are nonrandom and do not correlate with initial capillary lodgment (5, 9, 20, 21, 36, 37), suggesting that other factors are involved in specific malignant cell arrest.

During transport in the blood, malignant cells interact with each other as well as with host cells. Common intercellular interactions that affect the subsequent arrest and survival of malignant cells include homotypic adhesion of tumor cells to form multicell emboli (10, 27, 34), heterotypic adhesion of tumor cells to platelets (24), lymphocytes (14, 17), and noncirculating host cells (33, 34). In addition, malignant cell deformability (40, 48), release of hydrolytic enzymes (4, 41), and interactions with clotting components (8, 44, 46) and host immunological defenses (17, 23, 43) can determine whether tumor cells survive circulatory transport.

Experimental Models of Blood-borne Metastasis

Metastasis via the blood does not necessarily result in tumor colonization based on initial malignant cell distribution and circulatory pathways involved. Organ specificity of implantation, survival and growth of plasmacytoma (37), histiocytoma (9), melanoma (5, 20, 26), and reticulum cell sarcoma (36) suggests that patterns of malignant tumor colonization are nonrandom and uniquely dependent on specific tumor cell and host properties (22).

For studying the various steps in the metastatic process, it is desirable to have sets of metastatic and equivalent low metastatic tumor cells in order to directly compare properties important in metastasis. When variants of low and high metastatic potential are available, comparisons with dubious "normal" counterpart cells are unnecessary. Several of these tumor systems now exist (see below) where spontaneously, virally, and chemically transformed cell lines of low metastatic potential have been used to select variant lines of high metastatic potential and organ preference of colonization.

Lung-colonizing Melanoma Variants. In order to obtain malignant melanoma cell lines of differing metastatic potential, Fidler (11) used in vivo selection to enrich tumor cell populations with variants of high lung colonization abilities. Starting with an unselected murine B16 melanoma cell line of low in vivo metastatic potential, metastatic variants were selected for their abilities to implant, invade, survive, and grow to form gross lung tumor colonies after i.v. injection into syngeneic C57BL/6 mice. When pulmonary tumor nodules formed, these were identified by their melanin pigmentation and removed for adaptation to growth in culture. The B16 cells that grew in culture after the first in vivo selection were designated as line B16-F1, and these cells were harvested and injected i.v. into new groups of syngeneic animals to obtain the twice-selected line B16-F2.
After 10 selections, a line was obtained (B16-F10) which was more metastatic when compared to line B16-F1 in lung colonization (11) and spontaneous pulmonary metastasis assays (14).

The B16-F melanoma system has proven particularly useful for studying tumor cell and host properties associated with metastasis (22). Some advantages of the system are: (a) the tumor arose spontaneously in the skin and is maintained in syngeneic hosts; (b) tumors can be seen easily in the skin, lymph nodes, visceral organs, and at other sites because of their high melanin pigment content; and (c) tumor cells can be grown easily in tissue culture or at a variety of sites in vivo.

Lung specificity of line B16-F10 was assessed by following the kinetic distributions of \( ^{125}I \)-labeled melanoma cells after i.v. (tail vein) or left ventricle i.e.4 injection (20). By the latter route, extrapulmonary arrest, detachment, and recirculation are necessary in order to reach the lung microcirculation. Organs and blood from groups of animals were analyzed for viable \( ^{125}I \)-labeled B16 cells. Although the kinetic distributions of blood-borne melanoma cells were different in blood and all organs within 2 min after injection via these 2 routes, after 1 day the same numbers of viable B16-F10 cells were found in the lungs, independent of the route of melanoma cell entry into the circulation. When animals were sacrificed after 2 weeks and the numbers of gross melanoma nodules had been counted in the lungs and at other sites, the same numbers of pulmonary metastases were found in mice receiving melanoma cells via the tail vein (9 ± 3 and 79 ± 16 nodules for B16-F1 and B16-F10, respectively, per 25,000 input cells) or left ventricle (10 ± 2 and 83 ± 10 for B16-F1 and B16-F10, respectively, per 25,000 input cells) (20). B16-F10 extrapulmonary metastases were not found, indicating that initial blood-borne tumor cell distribution and arrest may have little bearing on subsequent metastatic colonization. Significantly more cells from the highly metastatic line B16-F10 implanted, survived, and grew to detectable lung metastases compared to line B16-F1 (11, 20), and B16-F1, but not B16-F10, cells formed a variety of extrapulmonary metastases (Table 1). Although the initial tumor cell distributions, rates of vascular entry, and first organ capillary beds encountered were different, the ultimate outcome of metastatic colonization was unchanged. Tumor cells entering into the circulation via the left ventricle and temporarily arresting in the extrapulmonary microcirculation were able to detach, recirculate, and ultimately reach the lungs, implant, and survive to form the same number of tumor colonies as melanoma cells entering the lung microcirculation first after tail vein injection. These data indicate that implantation and survival of blood-borne malignant cells are not random, and recirculation of once-arrested tumor cells can lead to efficient metastatic colonization (20, 32).

Arrest, detachment, recirculation, and rearrest of B16-F melanoma cells have been studied with the use of para-biosed pairs of syngeneic mice (21). After injection of \( ^{125}I \)-labeled B16-F cells into one animal of each pair, the kinetic distributions of viable melanoma cells were assessed in injected as well as uninjected parabiotic animals. These results indicated that viable blood-borne B16-F1 tumor cells temporarily arrested, recirculated, and traversed the site of circulatory anastomosis within 1 to 3 hr postinjection eventually to form pulmonary and extrapulmonary tumors in either parabiotic pair of animals. The kinetic distributions of viable B16-F cells and the number of experimental metastases that formed 2 weeks after injection in the injected parabiont were the same as in the nonparabiotic animals; in contrast, the kinetic distributions in the uninjected parabionts indicated that circulating tumor cells arrested and survived at lower efficiency in the lungs (B16-F1) or failed to be arrested and survive in the lungs (B16-F10). This indicates that selection for lung colonization leads to a decrease in detachment and recirculation of tumor cells destined to form metastases. Although B16-F10 cells were able to arrest, detach, and recirculate from injected to uninjected parabionts, these cells fail to form lung tumors in the uninjected parabionts (21).

The stability of B16-F lines after prolonged cell growth in vitro under nonselecting conditions has recently been questioned (31). Our results suggest that the differences between B16-F10 and B16-F1 in ability to form gross lung tumors, while still significant, decrease with long-term culture (G. L. Nicolson and I. J. Fidler, unpublished data). It may prove necessary to start fresh cultures from early passaged cells or to repeat in vivo selection procedures to prevent loss of phenotypic properties in tissue culture. However, some metastatic variant systems seem to be quite stable in tissue culture (see description of lymphosarcoma system).

**Brain-colonizing Melanoma Variants.** Brain-colonizing B16-B melanoma variants have been obtained from line B16-F1 by sequential in vivo selection for brain implantation, survival, and growth (Chart 1; Refs. 5, 32). B16-F1 cells were injected into the left ventricle of syngeneic mice by i.e. injection, and the resulting rare brain tumors were recovered and cultured to obtain line B16-B1. The selections were repeated 3 more times i.e., and further selections were carried out following injection into the tail vein. After 7 selections for brain colonization, 2 types of brain tumors were found (Chart 1). One type tended to implant and grow in the meninges of the dorsal cerebrum, and the other was located in or near the rhinal fissure between the olfactory bulb and the cerebral cortex. The cell line from the meninges was designated B16-B7b, and the rhinal fissure line was designated B16-B7n. Animals given injections of line B7n, but not B7b, exhibited "neurological symptoms" such as staggering gait, head tilt, and poor balance and orientation. After 3 additional i.e. selections for brain colonization, lines B16-B10b and B16-B10n were obtained, and these melanoma lines maintained their brain regionalization specificities. When line B16-B10n was analyzed for brain specificity and neurological disorders, almost every animal given injections had brain metastases exclusively (Table 1), and animals with B10n tumors exhibited a high degree of neurological symptoms (5). In these latter experiments B16 melanoma cells were injected i.e. via the tail vein, requiring the B16 melanoma cells to initially pass through the pulmonary capillary bed and then recirculate in order to reach the brain vascular bed. All brains collected from mice given injections of B16-B10n had melanoma nodules in or near
Characteristics of Metastatic Variant Cell Lines

Table 1

Locations of experimental metastases found after i.v. injection of lung- or brain-selected melanoma variant lines

<table>
<thead>
<tr>
<th>Melanoma line</th>
<th>Lung</th>
<th>Brain</th>
<th>Thoracic cavity</th>
<th>Ovary</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16-F1 (lung)</td>
<td>9/9, 10/10</td>
<td>4/9, 3/10</td>
<td>6/9, 6/10</td>
<td>2/9, 2/10</td>
<td>Adrenal, 1/10</td>
</tr>
<tr>
<td>B16-F10 (lung)</td>
<td>10/10, 10/10</td>
<td>0/10, 0/10</td>
<td>0/10, 0/10</td>
<td>0/10, 0/10</td>
<td>none</td>
</tr>
<tr>
<td>B16-B10n (brain)</td>
<td>0/10, 0/10</td>
<td>10/10, 10/10</td>
<td>0/10, 1/10</td>
<td>none</td>
<td></td>
</tr>
</tbody>
</table>

Chart 1. Experimental design for selection in vivo for B16 variant lines with enhanced brain colonization.

the rhinal fissure (5); often these tumors extended along vascular pathways into the cerebral cortex. Extension into the cortex was not seen with B16-B1 or B16-B5 tumors. B16-B10n cells also metastasized to brain after s.c. implantation. This has been shown following the s.c. implantation of 100,000 B16-B10n cells into syngeneic mice. After 2 weeks the resulting tumor was resected, and at this time regional lymph nodes were palpable. Two additional weeks later metastases were found in the brain (K. W. Brunson and G. L. Nicolson, unpublished data).

Line B16-B10n has recently been cloned in vitro to determine whether it is composed of a uniform or heterogeneous tumor cell population. Ten such clones were examined for invasive properties, neurological symptoms, and brain specificities. Preliminary experiments indicated that all clones were metastatic to brain, but not all clones were grossly invasive, or caused observable neurological disorders. Two of 10 clones were classified as grossly "neurological," and these same 2 clones were also dramatically invasive in the brain. Although other clones could be classified as grossly invasive due to their extension into the brain cortex, not all of the grossly invasive clones caused observable neurological symptoms. These data suggest that tumor cell heterogeneity exists even in the highly selected B10n line.

Ovary-colonizing Melanoma Variants. Ovary-colonizing B16 melanoma variants have been selected from B16-F1 for enhanced blood-borne implantation, survival, and growth in ovaries. After 10 selections for ovarian colonization, line B16-010 was obtained which forms more gross ovarian tumors compared to B16-F1. Line B16-010 also forms some experimental metastases at sites other than ovary and does not appear to be as organ-specific as lines B16-F10 or B16-B10n. Cloning B16-010 may yield a highly specific line...

that will exclusively colonize ovary.

Liver-colonizing Lymphosarcoma Variants. Brunson and Nicolson (7) have used the RAW117 lymphosarcoma line, which forms solid hepatic tumors in BALB/c mice to select for enhanced liver colonization. After 10 sequential selections for liver colonization, a lymphosarcoma line (RAW117-H10) was obtained that formed approximately 200 to 250 times more gross liver tumor nodules than the parental line in comparable biological assays (Table 2) and displayed enhanced malignancy when assessed by time of host death. In the latter assay the approximate times for 50% host death after i.v. injection of 5,000 viable lymphosarcoma cells were 40, 16, and 8 days for lines RAW117 parental, RAW117-H5, and RAW117-H10, respectively (7). Although liver colonization potential increased with succeeding in vivo selections, the numbers of lymphosarcoma nodules in the lungs were low in parental and selected RAW117 lines (Table 2). The stability of the unselected and selected lymphosarcoma lines was examined by comparing cell lines passaged for only a short time in vitro (2 weeks after thawing from liquid nitrogen) with the same cell lines that had been grown in tissue culture for 3 months. Differences in liver colonization between RAW lines grown for brief or long periods in tissue culture were not found, indicating that these cell lines are stable during long-term growth in vitro.

Lung-colonizing Sarcoma Variants. Untransformed, density-inhibited BALB/c embryo cell lines such as 3T3 have been virally transformed with oncogenic viruses such as SV40 and MSV to SV3T3 and MSV3T3 lines, which are not density-inhibited for growth and after in vivo s.c. implantation or i.v. injection form vasoformative sarcomas (1, 3). An MSV3T3 line has been used to select lung-colonizing variants. After 18 in vitro selections, line MSV3T3-LN18 was obtained that yields more lung nodules compared to unselected lines. Thus, sarcoma virus transformed cell lines can also yield metastatic variants that colonize lung.

Lymphocyte-resistant Melanoma Variants. B16-F melanoma variant lines are susceptible to killing by host immunocytes (12). The in vitro killing of B16-F cells by cytotoxic lymphocytes has been used to select B16-F variants resistant to cell-mediated killing. By sequentially exposing B16-F lines to syngeneic immune lymphocytes and recovering the survivors and repeating the process 6 times (17), lymphocyte-resistant lines (designated B16-F11* and B16-F102*) were obtained from B16-F1 and B16-F10, respectively. These 4 lines have been injected s.c. or i.v. into nonimmune syngeneic hosts. Growth at s.c. sites was similar for all 4 lines; however, the incidences of experimental pulmonary metastases after i.v. injection were quite different. The lymphocyte-resistant lines B16-F11* and B16-F102* formed significantly fewer lung metastases when compared to their parental lines B16-F1 and B16-F10, respectively (17). In this system, host lymphocyte interaction with the B16-F lines appears to enhance rather than inhibit the formation of metastases. Although the mechanism of lymphocyte resistance is not known, the lymphocyte-resistant cells show decreased binding of immune lymphocytes in heterotypic adhesion assays (16).

Metastatic Cells and the Primary Tumor

The existence of clonal variability in the properties of in vivo selected tumor cell lines such as B16-B10n and the ability to select this line from a melanoma line that was selected once for lung colonization (Chart 1) suggest that the original tumor may have been heterogeneous with respect to its phenotypic properties. Either these highly metastatic variants preexist in the primary tumor population or, alternatively, the selected lines could have arisen by the process of organ adaptation during the selection process. Cloning and fluctuation tests have been performed with the use of B16-F1 to determine whether highly metastatic variants preexist in the parent population (19). The cloned B16 cell lines differed significantly in their metastatic potential. Subcloning experiments ruled out the possibility that the in vitro cloning procedures yielded the variations among the clones. The existence of phenotypic heterogeneity in the primary tumor cell population has been hypothesized by Nowell (35). Host selection pressures could allow the emergence of sublines with enhanced malignant potential, compared to that of the original tumor.

Phenotypic heterogeneity of metastatic properties within an unselected tumor cell population has also been found with vasoformative sarcomas such as MSV3T3-LN (Table 3). In this case, groups of BALB/c mice were given injections i.v. with in vitro cloned tumor cell lines, and the number of metastases after i.v. injection was counted. Eight-week-old BALB/c mice were given injections i.v. with 2 x 106 single, viable in vitro cloned cells. Mice were sacrificed 18 days later, and gross pulmonary tumor colonies were visualized by injections of an India ink-formalin solution, given to the animals i.t.

Table 3

Experimental metastases found after i.v. injection of MSV3T3-LN1 clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>No. of lung tumor colonies</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0, 0, 0, 1, 1, 2, 3, 4</td>
<td>1 (0-4)*</td>
</tr>
<tr>
<td>19</td>
<td>2, 3, 3, 4, 5, 6, 10, 17</td>
<td>5 (2-17)</td>
</tr>
<tr>
<td>24</td>
<td>15, 16, 20, 21, 24, 27, 28, 29</td>
<td>24 (15-29)</td>
</tr>
<tr>
<td>37</td>
<td>24, 30, 34, 36, 42, 46, 49, 59, 66</td>
<td>42 (24-66)</td>
</tr>
<tr>
<td>45</td>
<td>18, 20, 33, 34, 49, 55, 63, 70</td>
<td>49 (18-70)</td>
</tr>
<tr>
<td>58</td>
<td>12, 15, 25, 28, 52, 54, 61, 69</td>
<td>52 (12-69)</td>
</tr>
<tr>
<td>55</td>
<td>16, 26, 36, 42, 55, 72, 74, 75, 79, 87</td>
<td>55 (16-87)</td>
</tr>
<tr>
<td>66</td>
<td>31, 42, 50, 55, 56, 57, 59, 64, 68</td>
<td>56 (31-68)</td>
</tr>
<tr>
<td>14</td>
<td>31, 50, 53, 57, 59, 62, 68, 73, 86</td>
<td>59 (31-86)</td>
</tr>
<tr>
<td>56</td>
<td>106, 111, 121, 130, 139, 141, 145, 155, 162</td>
<td>139 (106-162)</td>
</tr>
</tbody>
</table>

| Parent | 104, 107, 110, 111, 113, 125, 125, 127, 138, 143, 155, 162, 168, 194, 195, 198 |

* Numbers in parentheses. range.

4108
Characteristics of Metastatic Variant Cell Lines

Tumor Cell Surface Properties and Metastasis

A variety of cell surface changes have been correlated with transformation or tumor formation (25, 29, 38). Although some of these altered properties may be important in metastasis, little is known about tumor cell surface characteristics that determine malignancy and metastatic spread (22, 29). With the use of the metastatic variant lines described in preceding sections, it should be possible to determine tumor cell properties associated with successful blood-borne metastasis (18, 22).

The lactoperoxidase-catalyzed iodination procedure (28) has proven to be valuable in detecting cell surface modifications on metastatic cells. By this procedure, exposed cell surface proteins are 125I-iodinated, and the labeled proteins are separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Autoradiograms made from slab gels of separated B16-F1 and B16-F10 components revealed few differences, except in some minor protein bands at low molecular weight (31). However, cell surface proteins on brain-selected B16-B10n cells had identical autoradiograms, except for 2 prominently labeled components of 95,000 and 100,000 molecular weight which incorporated more 125I on the brain-colonizing lines (5). Alternatively, ovary-selected B16-O10 cells did not possess modifications in components with molecular weights of 95,000 and 100,000 but instead showed increased exposure of surface proteins with molecular weights of approximately 140,000 and 150,000, compared to those of unselected B16 melanoma lines. The degree of exposure to lactoperoxidase-catalyzed iodination of 95K and 100K components on brain-selected and 140K and 150K components on ovary-selected B16 melanoma lines correlated with enhanced metastatic potential. Cell surface changes detected by lactoperoxidase-catalyzed iodination have also been seen on in vivo selected RAW lymphosarcoma. Unselected RAW117 parental cells possessed a lactoperoxidase accessible protein with a molecular weight of 70,000, and this component was barely detectable on liver-selected RAW117-H10 cells. Other components of higher (~250K) and lower (~30K) molecular weight were also modified to a lesser degree in the liver-selected RAW lines (6). These results indicated that subtle changes occur on the cell surfaces of the selected metastatic lines; however, there was no direct proof that the changes in lactoperoxidase iodinability were directly related to tumor cell properties required for specific organ colonization.

Preferential in vivo localization of the selected tumor cell lines to certain organs suggested that adhesive interactions could lead to arrest in the microcirculation at specific locations. This might have been due, in part, to homotypic (self) adhesion between the circulating blood-borne tumor cells, because homotypic adhesion assays for lung-selected B16 melanoma (34, 35) and MSV3T3 sarcoma (30) indicated that the highly metastatic lung-colonizing lines undergo in vitro homotypic adhesion at faster rates. The formation of multicellular tumor emboli is known to enhance experimental pulmonary colonization. This was shown by Fidler (10), who purposely aggregated B16 melanoma cells and then injected the aggregates i.v. and scored for lung tumor formation. In these experiments the cells within the larger tumor cell emboli implanted and survived at significantly greater rates compared to small emboli or single cells. In addition, heterotypic adhesions of B16-F melanoma cells with platelets (24), lymphocytes (14), and endothelial cell lines (34, 45) probably affected rates of tumor cell arrest in the microcirculation.

Organ preference in arrest and colonization could be explained by adhesive interactions of circulating tumor cells with noncirculating host cells in the target organ (33). In an experimental approach to this problem, B16-F cells have been mixed with partly purified single target or nontarget organ cells in suspension, and heterotypic aggregation was determined within 5 min. In vivo selected B16-F lines such as B16-F10 and B16-F13 aggregated rapidly with suspended lung cells in vitro, but only slowly with nontarget organ cells (kidney, spleen, liver, etc.) in similar experiments (22, 33). Thus, cell-to-cell recognition and adhesion may play an important role in directing tumor cell implantation at specific organ sites.

Tumor cell invasive properties have been used to characterize malignant neoplasms, and the selected metastatic tumor cell lines that have been placed into in vitro invasion assays have shown an increased ability to invade normal tissue matrix, compared to their unselected parental counterparts. This has been experimentally demonstrated with the use of the chorioallantoic membrane as an invasion substrate in tissue culture. When B16-F1 and B16-F10 were compared for their ability to infiltrate and invade the several cell-thick chick embryo chorioallantoic membrane, only the selected B16-F10 cells invaded within 1 day, while the B16-F1 cells were found loosely attached to the chorioallantoic membrane surface (31). Although other in vivo selected tumor cell lines have not been compared with the use of these techniques, in vivo metastatic selected lines also appear to be more invasive in syngeneic hosts when injected s.c. (Ref. 14; I. J. Fidler and G. L. Nicolson, unpublished data).

Invasion of malignant cells into surrounding normal tissues is thought to occur by means of a combination of mechanical and enzymatic mechanisms (for review, see Refs. 18 and 22). Release or display of degradative enzymes may be important in invasion, because normal intercellular tissue matrix and similar structures are sensitive to enzymatic destruction (2). In the B16-F system in which degradative enzymes have been measured, Bosmann et al. (4) found that the more metastatic B16-F10 line in vitro had slightly higher glycosidase and protease (trypsin- and cathepsin-like) activities when compared to those of B16-F1, but only when the cells were replated at low density in vitro...
tissue culture. Other enzyme activities such as plasminogen activator, a released serine proteinase that converts blood plasminogen to plasmin, which in turn hydrolyzes fibrin, do not directly correlate with metastatic potential (34).

Some immunological properties of the selected metastatic tumor cell lines have been examined. Lung-selected B16-F melanoma cell lines carry a variety of normal and tumor-associated antigens, as well as receptors for cytoxic lymphocytes, but dramatic changes in the expression of these surface determinants have not been detected on highly selected metastatic B16-F lines (22, 31). Interestingly, liver-colonizing RAW117 lymphosarcoma lines apparently have almost lost gp 71, the major RNA tumor virus envelope glycoprotein, after 8 to 10 in vivo selections for liver colonization (6). In this case a dramatic reduction in anti-gp 71 binding correlated with the loss in exposure of surface protein with a molecular weight of 70,000 detected by lactoperoxidase-catalyzed iodination.

The immunological properties of lymphocyte-resistant B16-F10^-c^- cells suggested that these lines have lost receptors for immune lymphocytes. The resistance of the lymphocyte-selected B16-F1^-st^- lines to lysis by syngeneic lymphocytes was not associated with loss of all antigens such as H-2, and these tumor cells remained sensitive to killing by allogeneic lymphocytes and syngeneic or allogeneic macrophages (22). B16-F10^-c^- cells did not protect against lymphocyte-mediated cytotoxicity of lymphocyte-sensitive B16-F10 cells in coculture experiments, indicating that resistance was not due to release of "blocking" factors by the B16-F10^-c^- cells (16). Also, immunization of mice with B16-F10^-c^- did not protect against challenge with B16-F10, although immunization with B16-F10 protected against subsequent B16-F10 challenge. In vitro tumor cell-lymphocyte aggregation experiments have demonstrated that resistance to lymphocyte cytotoxicity is associated with decreased lymphocyte binding (16, 17, 22). Collectively, these experiments suggest that the lymphocyte binding sites could be tumor-associated transplantation antigens and that these are altered or missing on the B16-F-1^-c^- cell lines (22).

Conclusion

The spread of solid tumors via blood-borne metastasis usually marks the final, terminal stage of the pathogenic sequence of malignant neoplasms, and the complex series of interactions between tumor cells and their host is of paramount importance in this process. The unique properties of malignant tumor cells determine, in part, whether they are able to invade, enter the blood, detach, circulate, arrest, survive, and grow to form secondary tumor colonies. Few tumor cells in the initial population are probably able to successfully continue at each step in the metastatic process, and this selective pressure undoubtedly results in enrichment of cells that possess unique properties in the highly metastatic cell lines. In addition to unique tumor cell characteristics, host properties such as immune response can influence metastasis. The role of host immune antitumor response in metastasis has been reviewed elsewhere (18, 22). It suffices to mention here that both tumor cell and host properties probably determine whether metastasis occurs in each tumor-host system.

References

29. Nicolson, G. L. Transmembrane Control of the Receptors on Normal and
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