Persistence of Immunogenic Pulmonary Metastases in the Presence of Protective Anti-melanoma Immunity

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ABSTRACT

We have developed a murine melanoma model that allows us to investigate the mechanisms by which spontaneous, immunogenic melanoma metastases escape immunological destruction in syngeneic mice. In the current study, we tested the hypothesis that loss of immunogenicity is an obligatory step in the persistence of pulmonary metastases. Fragments of syngeneic K1735-M2 tumor were implanted in the outer edge of one pinna per C3H/HeN mouse, and the growing tumors were removed 2–3 weeks later. Two weeks after removal of the tumors, the mice demonstrated effective T-cell-mediated immunity to s.c. challenge with K1735-M2 cells. However, lung metastases appeared in 23% of the immunized mice within 9–12 weeks after the initial tumor implantation. The expression of protective immunity to s.c. tumors required the presence of both CD4+ and CD8+ T cells. The immunized mice had specific CTLs capable of killing both K1735-M2 melanoma cells and the cells of nine independently derived melanoma metastases. Furthermore, K1735-M2 immunization protected these mice from s.c. tumor challenge with all nine metastatic cell lines. Our results demonstrate that the persistence of these metastases within the lung was not attributable to emergence of antigen-loss variants in immunized hosts. Our model provides an approach to investigate other mechanisms by which spontaneous metastases escape from immunological control and an opportunity to improve immunotherapy of melanoma metastases.

INTRODUCTION

Because of early clinical observations suggesting that cutaneous melanomas are immunogenic, the immunotherapy of human cancer initially focused on malignant melanoma. Spontaneous regression of cutaneous melanoma lesions in humans is well documented, and such regressions are often associated with lymphocytic infiltrates suggestive of an immunological reaction (1, 2). The extent of lymphocytic infiltrate in a vertical growth phase melanoma is a prognostic indicator of long-term survival (3). In addition, studies in patients of both humoral and cellular immune responses against either their autologous melanoma or allogeneic melanoma cells indicate that melanomas are often immunogenic (4–7). In other experimental studies, melanomas have been shown to produce a variety of immunologically active cytokines that could down-regulate an immune response within their microenvironment (8–11).

Although no single immunotherapeutic approach for treatment of melanoma has cured the majority of patients (4, 6, 12–15), immunization with vaccines in both clinical trials and experimental observations (4, 16–18) have provided suggestions that immunotherapy can cure or control disease in a minority of patients.

In experimental studies, Sabzevari et al. (19) reported that human melanoma cells implanted s.c. in SCID mice spontaneously metastasized to the lungs. If these mice were given i.v. injections of human cytotoxic T cells after s.c. tumor implantation, the mean number of lung metastases was reduced from 115 to less than 1 metastasis/mouse. Although this was an impressive reduction, seven of eight mice treated with human CTLs still had 1 metastasis in the lung. This study illustrates the clinical problem that frequently occurs in many immunotherapy trials; significant tumor eradication is attained, but a single residual metastasis ultimately kills the patient (16, 20, 21). Early experimental studies indicated that tumors make antigen-loss variants, which could then be responsible for spontaneous metastases (22, 23). Recent clinical evidence however, indicates that in many instances other factors may be more important than antigen loss for the success of distant metastasis. Organ site and/or the selective expression of immunity within the particular organ may play important roles in successful expression of specific immunity against immunogenic metastases (24–26).

We have used the spontaneously metastatic K1735-M2 murine melanoma (27) to develop a model to investigate approaches for controlling immunogenic pulmonary metastases. In early experiments, we observed that immunity to s.c. tumor challenge failed to correlate with protection against spontaneous metastases in the lungs of mice. The model, therefore, provides an excellent opportunity to determine the basis for the failure of an active, systemic immune response to prevent the formation of or to eradicate spontaneous lung metastases. In this study, we tested the hypothesis that the escape of spontaneous lung metastases from immunological control resulted from antigen loss in the metastases. We demonstrate that the metastases maintained their antigenic identity and immunological reactivity, and therefore, antigen loss did not account for their success.

MATERIALS AND METHODS

Experimental Animals. C3H/HeN (MTV–), specific pathogen-free female mice were housed five per cage and maintained on a diet of sterile water and NIH open formula pellets. Ambient light was regulated on a 12-h light-dark cycle. The mice were housed in a specific pathogen-free facility accredited by the American Association of Laboratory Animal Care under conditions that meet or exceed the standards set by the United States Department of Agriculture Animal Welfare Act, Public Health Service policy on humane care and use of animals, and the NIH guide on laboratory animal welfare. The Institutional Animal Care and Use Committee approved all experimental protocols.

Tumor Cell Lines. K1735 was derived from a melanoma induced in C3H/HeN (MTV–) mice by ultraviolet radiation initiation and croton oil promotion (28). The K1735-M2 subline was developed by repeated selection from murine pulmonary metastases in Dr. I. J. Fidler’s laboratory (27). The HCA tumor cell line is a spontaneous hepatocarcinoma from a C3H/HeN mouse generously provided by Dr. Luka Milas (Department of Experimental Radiation Oncology, University of Texas M. D. Anderson Cancer Center), and the YAC-1 line is an NK-sensitive mouse lymphoma obtained from the American Type Culture Collection (Rockville, MD).

We established cell lines from nine individual pulmonary metastases (1CD98, 3CD98, 4CD98, 6CD98, 9CD98, 10CD98, 2CD92, 5CD92, and 8CD92) recovered from syngeneic mice implanted s.c. with K1735-M2 tumors. These cells were grown in tissue culture for use in cytotoxicity assays.

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4 The abbreviations used are: NK, natural killer; DTH, delayed-type hypersensitivity; LATA, local adoptive transfer assay; mAb, monoclonal antibody.
and for in vivo s.c. tumor challenge experiments. All tumor cell lines were maintained in Eagle’s MEM supplemented with 10% fetal bovine serum, vitamin solution, t-glutamine, sodium pyruvate, and nonessential amino acids, without antibiotics. They were harvested by 1–2 min incubation in 0.25% trypsin-0.02% Versene (edetate sodium) solution. The cells were washed three times and resuspended in Eagle’s MEM buffered with HEPES buffer solution for injection into mice. All cell lines were routinely tested for and found free of Mycoplasma and pathogenic murine viruses.

**Immunization and Spontaneous Metastasis Protocol.** K1735-M2 cells were injected s.c. in either C3H/SCID mice or C3H mice immunosuppressed by thymectomy at 6 weeks of age and exposure to 450 cGy of X-irradiation 24 h before tumor cell injection (29). The tumors were resected 3–4 weeks after tumor cell injection and cut into 1-mm³ fragments. These fragments were implanted into the pinnae of normal C3H mice. After 2–3 weeks of tumor growth (when tumors were 4–6 mm in diameter), the tumors were removed by excision of the ear. These mice were considered immunized in vivo and were then used for tumor challenge or observed for the formation of lung metastases. Sham immunization involved all aspects of the anesthesia and surgical procedures except the actual implantation of tumor tissue.

**Tumor Challenge Protocol.** Two to 3 weeks after the removal of the tumor from the pinnae, mice were challenged by s.c. injection of melanoma cells into the remaining pinnae in a 50-μl volume. Mice were numbered and examined weekly for tumor growth. Tumors were measured in two bisectional diameters with a caliper. Moribund mice were euthanized by cervical dislocation under CO₂ sedation, and their lungs were removed for gross and microscopic evaluation of metastases. The metastases were recovered for growth in tissue culture and for use in various in vivo and in vitro assays.

**In Vitro Assay for Cytotoxic T Cells.** Two to 3 weeks after the removal of the immunizing tumor (see immunization procedure above), the mice were boosted once with an additional injection of 2 × 10⁶ γ-irradiated K1735-M2 tumor cells in DETOX™ adjuvant specially formulated for mice (RIBI ImmunoChem Research, Inc., Hamilton, MT). One week later, mice were sacrificed, and the recovered spleen cells (5 × 10⁶/ml) were placed into culture with 1 × 10⁹/ml γ-irradiated K1735-M2 tumor cells in 24-well plates (2 ml/well) for 5 days in RPMI 1640 with 10% FBS, supplements, and antibiotics (29). After 5 days, the nonadherent cells were collected and used as effectors with 51Cr-labeled tumor target cells at 25:1, 50:1, 100:1, and 200:1 ratios in 96-well round-bottomed plates. The plates were incubated at 37°C for 6 h, supernatants of the cultures harvested, and 51Cr release was counted by a gamma counter. Cells in control wells were lysed with 1% SDS to obtain the total 51Cr release. The results are expressed as the arithmetic mean of the percentage of specific 51Cr release of triplicate cultures; the spontaneous lysis of target cells was ≤15%. The percentage of specific 51Cr released was calculated as follows:

\[
\% \text{ specific } 51\text{Cr release} = \frac{\text{Experimental (cpm) } - \text{ spontaneous (cpm)}}{\text{Total release (cpm) } - \text{ spontaneous (cpm)}} \times 100
\]

**Induction and Measurement of DTH.** Two to 3 weeks after the immunizing tumor was removed (see immunization procedure above), the mice were boosted once with an injection of 2 × 10⁶ γ-irradiated K1735-M2 tumor cells into the remaining pinnae in a 50-μl volume. Mice were numbered and examined weekly for tumor growth. Footpad swelling was measured 24 h after challenge.

**LATA.** Mice were immunized as above; 2 weeks after the removal of the implanted tumor, the mice were sacrificed. Lymph nodes and spleens were recovered, and cells from these organs were mixed with viable tumor cells for s.c. injection into naive recipient mice. The spleen cells (3.75 × 10⁵/50 μl) were mixed with K1735-M2 melanoma (1 × 10⁵/50 μl) cells at a (375:1) spleen:tumor cell ratio, and the lymph node cells (7.5 × 10⁵/50 μl) were mixed with K1735-M2 melanoma (1 × 10⁵/50 μl) cells at a 75:1 ratio. The 100-μl mixtures were then injected into the ears of normal syngeneic C3H mice, and tumor growth was monitored weekly.

**In Vivo Depletion of T-Cell Subsets.** mAbs GK1.5 (anti-CD4; rat IgG2b) and 116–13.1 (anti-CD8; mouse IgG2a) from the American Type Culture Collection (Rockville, MD) were used to deplete CD4+ and CD8+ T-cell subsets by injecting them in vivo. The hybridomas GK1.5 and 116–13.1 were grown either in protein-free medium (PSPH-II GIBCO) and concentrated 10× using an Amicon concentrator (Amicon, Inc., Beverly, MA) or in CMEM with 5% FBS and then concentrated by ammonium sulfate precipitation, followed by dialysis in sterile PBS at 4°C for 24 h. The absence of endotoxin was determined with the PYROTELL Limulus amebocyte lysate assay (Associates of Cape Cod, Inc., Falmouth, MA). The mAb preparations were then titered against C3H lymphocytes and evaluated by flow cytometric analysis (fluorescence-activated cell sorter) with the Coulter Epics Profile (Coulter Corp., Miami, FL).

To determine the in vivo biological activity of the anti-CD4 and anti-CD8 mAbs, mice were injected i.p. three times per week (Monday, Wednesday, and Friday) with either 100 μl (160 μg of protein) of anti-CD4, 100 μl (46 μg of protein) of anti-CD8, or 100 μl (200 μg of protein) of rat IgG. Each week for the next 5 weeks, lymph nodes were recovered from two to four mice and pooled for each treatment group; then these lymph node cells were double-stained with anti-CD4-PE (phycoerythrin) and anti-CD8-FITC (fluorescein) so that the percentage of positive cells could be determined by two-color fluorescence-activated cell sorter analysis.

**Statistical Analysis.** Differences in tumor incidence between treatment and control groups were analyzed with the Kaplan Meier Survival/Log Rank statistics. In the DTH assay, differences between groups were analyzed with ANOVA.

**RESULTS**

**Induction of Immunity against s.c. Challenge Fails to Protect against Spontaneous Pulmonary Metastases.** We implanted fragments of K1735-M2 tumor, followed by removal of the tumor after 3 weeks of growth. When these mice were s.c. tumor challenged with K1735-M2 tumor cells 2–3 weeks later, this procedure produced significant protection against the outgrowth of the K1735-M2 tumor challenge. A summary of the immunization and challenge data for six separate experiments (Fig. 1) shows that although 40% of the immunized mice developed tumors upon challenge, 91% of the nonimmunized control mice developed tumors; therefore, there was 51% protection against tumor challenge in tumor-implanted, resected (immunized) mice. However, 23% of the immunized mice that survived the s.c. tumor challenge eventually died from pulmonary metastases arising from the initial tumor implant.

Table 1 shows the occurrence of spontaneous metastases in mice from the six experiments summarized in Fig. 1. Although immunization with K1735-M2 cells protected 51% of the mice from an otherwise lethal s.c. tumor challenge, 23% of these successfully protected...
mice died 3–6 weeks after tumor challenge from spontaneous lung metastases. The incidence of pulmonary metastases in immunized, tumor-challenged mice (23%) was not significantly different from the incidence of pulmonary metastases (19%) in immunized, nonchallenged mice. That these metastases must have originated from the primary tumor implant is supported by the observation that only the previously implanted (immunized) mice but none of the tumor-challenge-only control mice developed pulmonary metastases within this time period.

The failure to develop macroscopic metastases from the tumor challenge inoculum was probably attributable to the death of the mice from the large s.c. challenge tumor, before visible lung metastases were apparent. The time needed for spontaneous lung metastases of 2 mm in diameter to develop was 9–12 weeks after implantation of the initial tumor. The tumors from the s.c. challenge grew to 15 mm in diameter within 5–8 weeks of tumor challenge, and these mice were sacrificed (data not shown); therefore, there was insufficient time for spontaneous lung metastases arising from the s.c. tumor challenge to be detected. Finally, the strongest evidence that the metastases arose from the initial implants was that in all mice previously implanted an equal percentage of lung metastases occurred, regardless of subsequent s.c. tumor challenge. Thus, the systemic immune mechanisms capable of rejecting s.c. tumors appeared to have no detectable influence on the development of pulmonary metastasis in these mice. Therefore, we hypothesized that the spontaneous lung metastases must be escaping immune destruction by either loss of immunosensitivity (30) or by evasion of T cell-mediated immunity by other mechanisms (10, 31, 32).

**Immunogenicity of Lung Metastases**

**Table 1** Incidence of s.c. tumors and spontaneous pulmonary metastases in K1735-M2-immunized mice

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Immunized</th>
<th>Challenge</th>
<th>Incidence of s.c. tumor</th>
<th>% of mice protected from tumor challenge</th>
<th>Incidence of pulmonary metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>All mice</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>–</td>
<td>5/9 (56%)*</td>
<td>44</td>
<td>8/13 (62%)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>+</td>
<td>10/10 (100%)</td>
<td></td>
<td>6/9 (67%)</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>–</td>
<td>13/19 (68%)*</td>
<td>32</td>
<td>2/14 (14%)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>+</td>
<td>15/15 (100%)</td>
<td></td>
<td>3/17 (18%)</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>–</td>
<td>5/23 (22%)*</td>
<td>78</td>
<td>1/23 (4%)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>+</td>
<td>18/18 (100%)</td>
<td></td>
<td>4/22 (18%)</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>–</td>
<td>4/23 (17%)*</td>
<td>63</td>
<td>3/23 (13%)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>+</td>
<td>12/15 (80%)</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>–</td>
<td>14/23 (61%)*</td>
<td>29</td>
<td>4/17 (24%)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>+</td>
<td>9/10 (90%)</td>
<td></td>
<td>1/22 (5%)</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>–</td>
<td>4/5 (27%)*</td>
<td>43</td>
<td>1/11 (9%)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>+</td>
<td>7/10 (70%)</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Totals</td>
<td>+</td>
<td>–</td>
<td>45/112 (40%)*</td>
<td>51</td>
<td>19/101 (19%)</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>+</td>
<td>71/78 (91%)</td>
<td></td>
<td>14/62 (23%)</td>
</tr>
</tbody>
</table>

* K1735-M2 cells were injected into adult thymectomized, sublethally X-irradiated mice. 3 weeks later, the tumors were resected, and 1-mm³ fragments were implanted into normal C3H mice. After 3 weeks, these tumors were removed; 3 weeks later, they were challenged with 1 × 10⁵ K1735-M2 cells in the remaining pinnae. Mice were evaluated for s.c. tumor growth weekly and for metastases when moribund in the immunized groups. Incidence data are from the 4th week after tumor cell challenge.

* P ≤ 0.001 throughout the experiment versus nonimmunized mice.
* P ≤ 0.01 at one or more time points within the experiment versus nonimmunized mice.

Specifically, 5CD92, 3CD98, and 10CD98 cells had; however, there was measurable killing, even at passages 1–5, as shown in Fig. 3. No evidence was found that any of these metastasis-derived cell lines in vivo.
Fig. 2. K1735-M2-immunized mice demonstrated in vivo cross-protective immunity to s.c. tumor challenge with early-passage, metastasis-derived cell lines. Mice were immunized by implanting the parental K1735-M2 tumor fragment, followed by resection after 2–3 weeks. Then 2 weeks later, K1735-M2-immunized mice and nonimmunized controls were tumor challenged with injection of either 2 × 10^5 parental K1735-M2 tumor cells or metastases-derived cells into the remaining pinnae. A, K1735-M2-immunized and K1735-M2-challenged (●), K1735-M2 challenged only (○), K1735-M2-immunized and 6CD98-challenged (■), and 6CD98-challenged only (□); *P < 0.003; †P ≤ 0.0001; n = 20–29. B, K1735-M2-immunized and K1735-M2-challenged (●), K1735-M2-challenged only (○), K1735-M2-immunized and 9CD98-challenged (■), and 9CD98-challenged only (□); †P ≤ 0.003; †P ≤ 0.01; n = 20–29. C, K1735-M2-immunized and K1735-M2-challenged (●), K1735-M2-challenged only (○), K1735-M2-immunized and 5CD92-challenged (■), and 5CD92-challenged only (□); *P < 0.003; †P ≤ 0.0001; n = 16–19.

Expression of DTH Correlates with s.c. Tumor Challenge Rejection. Because patients treated with polyvalent melanoma cell vaccine have a significant correlation between increased DTH reactions to their tumor cells and improved survival (4–6), the DTH response to K1735-M2 melanoma cells and its relationship to the expression of in vivo s.c. tumor immunity was assessed. Mice were immunized with γ-irradiated K1735-M2 tumor cells mixed with the adjuvant DETOX™ and then challenged with γ-irradiated K1735-M2 tumor cells. Two weeks after testing their DTH response, we produced a s.c. tumor challenge by injecting the mice with viable 2 × 10^5 K1735-M2 melanoma cells in one pinna. Mice were considered successfully immunized when the s.c. tumor challenge was rejected and unsuccessfully immunized when the challenge tumors grew. As shown in Fig. 4, the successfully immunized mice made a substantially greater DTH response (141% increase) compared with the DTH response of unsuccessfully immunized mice prior to the s.c. tumor challenge. This result indicates that a strong T cell-mediated DTH response correlated with the successful expression of K1735-M2 tumor immunity in vivo, as measured by tumor rejection, and was prognostic of effective immunization.

We investigated whether the K1735-M2-immunized mice could generate tumor-specific CTLs. The specificity of these CTLs was evaluated using three target cells: K1735-M2 melanoma cells; the YAC-1 cell line (an NK-sensitive target cell); and a syngeneic C3H hepatocarcinoma HCA. As shown in Fig. 5, specific CTL effectors capable of lysing K1735-M2 tumor cells were detected in vitro. No significant activity was detected against the YAC-1 targets or the HCA cells. The results of the DTH and CTL experiments indicate that immunization with K1735-M2 elicited specific and effective T cell-mediated immunity against K1735-M2 tumor.

In Vivo Immunity to K1735-M2 Melanoma Is T-Cell Dependent. To further define the immunological mechanisms that provided s.c. tumor immunity in these K1735-M2-immunized mice, we took two additional approaches. A LATA was performed, using the spleen and lymph node cells recovered from K1735-M2-immunized mice, mixed with viable K1735-M2 melanoma cells and then injected into the pinnae of nonimmunized, syngeneic mice. As illustrated in Fig. 6, both spleen and lymph node cells from K1735-M2-immunized mice were capable of significantly reducing the outgrowth of K1735-M2 in the LATA. This result indicated that lymphoid cells transferred tumor resistance from immunized mice.

Because lymphoid cells were responsible for tumor protection, the role of T-cell subsets in the expression of in vivo K1735-M2 tumor immunity was determined by depletion of either CD4+ or CD8+ T-cell subsets with monoclonal anti-CD4 or anti-CD8. Previously immunized (by implantation and resection) mice were treated with i.p. injections of anti-CD4, anti-CD8, or rat IgG three times in 1 week. We accomplished >90% depletion of the CD4+ and CD8+ T-cell subsets with this approach (data not shown). The 90% depletion of either CD4+ or CD8+ T cells was sufficient to completely abrogate expression of in vivo s.c. tumor immunity (Fig. 7). These experiments confirmed that the presence of both CD4+ and CD8+ T cells was required during tumor challenge for successful expression of K1735-M2 tumor immunity in vivo. Therefore, we determined that specific T cell-mediated immunity was protective in vivo against s.c. tumor challenge.

DISCUSSION

The K1735-M2 murine melanoma model has many features that correlate closely with the pathogenesis of human melanoma. This melanoma cell line develops into a well-vascularized tumor when injected in the pinnae of mice. Even when this tumor reached a modest size of 4–6 mm in diameter, it remained resectable, because no regrowth occurred at the site of resection. However, in ~20% of these mice, the tumor gave rise to spontaneous pulmonary metastases that subsequently killed them.

Mice exposed to viable tumor demonstrated specific T cell-mediated immunity after removal of the growing tumor. Both lymph node and spleen cells were capable of preventing the outgrowth of s.c. injected tumor cells. Furthermore, both CD4+ and CD8+ T cells are required to protect the mice against s.c. tumor challenge. These mice had enhanced DTH responses to tumor cells that correlated with in vivo protection against s.c. tumor challenge. Immunized mice gener-
The inability to control previously established metastases by immunological approaches that are highly effective against s.c. tumor challenge is a well-known phenomenon in animal model systems. Generally, such failures have been attributed to the problem of “tumor burden,” which implies that metastases grow so rapidly that they overcome immune mechanisms (35–37). However, this interpretation is not supported by our experiments, because the 23% of immunized mice that had pulmonary metastases were also able to reject s.c. tumor challenge in the presence of these metastases.

Another possible explanation for the escape of lung metastases is the compartmentalization of the immune system. In recent years, it has become clear that different organs of the body have different and highly specialized immunological capabilities. For example, the immunological approaches that are highly effective against s.c. tumor challenge in mice becomes largely exclusive, and an individual metastasis may escape immune destruction by more than one mechanism. Although the development of pulmonary metastases in the presence of an antitumor immune response can result from the surviving metastatic cells becoming antigen-loss variants, this is only one possible explanation for their immune escape (22). This phenomenon has been described during the s.c. passage of antigenic tumors in immunocompetent hosts (23), and there is evidence for decreased antigenicity of metastases compared with the primary tumor in various tumor systems (22). However, our results indicated that in none of the nine pulmonary metastases tested was the loss of immunogenicity the primary method of escape from the immunity expressed against the s.c. tumor.

It should be noted that metastases as a group are heterogeneous with regard to many phenotypic properties (34); therefore, it is unlikely that all metastases escape immune control by the same mechanism. Furthermore, the potential escape mechanisms are not mutually exclusive, and an individual metastasis may escape immune destruction by more than one mechanism. Although the development of pulmonary metastases in the presence of an antitumor immune response can result from the surviving metastatic cells becoming antigen-loss variants, this is only one possible explanation for their immune escape (22, 23). This phenomenon has been described during the s.c. passage of antigenic tumors in immunocompetent hosts (23), and has become clear that different organs of the body have different and highly specialized immunological capabilities. For example, the immunological approaches that are highly effective against s.c. tumor challenge (35–37).

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immune apparatus and capabilities of the skin, which is efficient at mounting DTH responses, are quite different from those of the gut, which is designed for making antibody (38). Therefore, the immune mechanisms that are highly efficient at destroying melanoma cells in the skin may be quite ineffective against melanoma cells growing in the lungs. More important, the ability of lymphocytes to home to and provide effective immunity within a particular organ is determined by their expression of specific receptors for molecules on the endothelial cells of the organ in question (39–41).

It is clear that the organ environment profoundly influences the characteristics of tumor cells and their susceptibility to therapy (34, 42–44). It is possible that melanoma cells growing in an internal organ respond to the specific organ microenvironment by up-regulating or producing cytokines that inhibit immune effector cells, whereas the same melanoma cells growing in skin may fail to express these inhibitory cytokines and are therefore susceptible to immunological destruction. In any case, immune selection does not appear to be a common mechanism by which this tumor evades immunological control.

In these studies, we have begun to explore, in a systematic way, the basis for the failure of immune mechanisms to control the growth of melanoma metastases in the lung. Because antigen loss is not an obligatory step in the escape of metastases from immunological control, other possible mechanisms will be investigated using our model. We now hypothesized that the spontaneous lung metastases must be escaping immune destruction by either loss of immunosen-sitivity in situ (30) or by evasion of specific T cell-mediated immunity by other mechanisms (10, 31, 32). Possibilities yet to be tested include down-regulation of HMC class I in situ, which could provide recognition of the metastases and result in protection from killing by specific T cells (32). Alternatively within the lung, the up-regulation of Fas ligand in the endothelial cells of the lung or in the tumor cells themselves could be responsible for selective killing of activated CD4+ T cells, thus preventing them from killing tumor cells (31). It is well established that human melanoma cells are capable of producing cytokines such as transforming growth factor-β or interleukin 10, both of which are able to down-regulate the immune cells within the microenvironment of pulmonary metastases (9–11).

In conclusion, in these experiments we have shown that immunogenic pulmonary metastases can survive in immune hosts by mechanisms other than antigen loss. The challenge now is to define the escape mechanisms in this model that allow metastatic melanoma to grow in the lung so that treatment strategies may be more successful in the eradication of metastases.

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REFERENCES

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