Nonselective Destruction of Murine Neoplastic Cells by Syngeneic Tumoricidal Macrophages

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ABSTRACT

The purpose of these studies was to select in vitro tumor cells that were resistant to macrophage-mediated lysis. Seven different heterogeneous murine neoplasms (four fibrosarcomas, a melanoma, a rhabdomyosarcoma, and an osteogenic sarcoma) and one cloned line of a fibrosarcoma were incubated in vitro with syngeneic tumoricidal macrophages. Surviving tumor cells were recovered and expanded to undergo subsequent interactions with tumoricidal macrophages. After six sequential interactions, all cell lines were examined for their susceptibility to lysis mediated by murine peritoneal exudate macrophages activated with liposomes containing muramyl tripeptide phosphatidylethanolamine. In all eight systems, no significant differences were detected between the parent tumor cells and cells that survived the sequential interactions. Neither macrophage infiltration into s.c. tumors nor the experimental or spontaneous metastatic potentials of the parental tumors differed from the lines established by cells surviving macrophage-mediated lysis. Collectively, the data suggest that tumor cell destruction by activated macrophages is nonselective and does not lead to the development of resistant tumor cells nor to cells with altered metastatic properties.

INTRODUCTION

Different metastases proliferating in the same or different organs can exhibit both inter- and intralesional heterogeneity with respect to many biological characteristics, including response to therapeutic agents (2, 8, 16). The interlesional heterogeneity may be due to the clonal origin of metastases and the origination of different metastases from different progenitor cells (31). The intralesional heterogeneity observed in many metastases could be due to the phenotypic instability of clonal populations (early metastasis) (24) and the increased rate of spontaneous mutation in metastatic cells as compared with nonmetastatic cells (3, 17). The corollary of these findings is that investigators devising therapeutic modalities against metastases must circumvent biological heterogeneity in metastases and prevent the emergence of treatment-resistant tumor cells.

Rodent (25, 29) and human (19, 20) macrophages activated by various immunomodulators, such as lymphokines or muramyl dipeptide, in either free form or entrapped within liposomes, acquire the ability to destroy neoplastic cells in vitro while leaving nonneoplastic cells unharmed, even under cocultivation conditions (19). The susceptibility of neoplastic cells to lysis by activated macrophages is independent of tumor cell characteristics such as antigenicity-immunogenicity (6), invasion and metastasis (4, 6, 9), growth rate (6, 11), and resistance to lymphocyte- (9) or NK3 cell-mediated (9) lysis. Of particular importance in the treatment of metastasis is the observation that tumor cell variants selected for resistance to the anthracycline antibiotic Adriamycin remain fully susceptible to destruction by tumoricidal macrophages (14).

Macrophages can be rendered tumoricidal in situ by the systemic administration of liposomes containing immunomodulators (reviewed in Ref. 9). Moreover, the continuous activation of macrophages by multiple i.v. injections of liposomes containing immunomodulators has been responsible for the eradication of spontaneous, well-established lung and lymph node metastases from melanomas growing in the subcutis of syngeneic mice (5, 12). This initial success of treating metastases in vivo by the systemic activation of macrophages raised the question of whether direct or indirect tumor cell resistance against this effector cell can emerge. For this reason, we wished to determine whether in vitro techniques used previously to select tumor cells resistant to lysis by T-cells (7) or NK cells (15) could also be used to select cells resistant to macrophage-mediated lysis. We present data demonstrating that macrophage-mediated destruction of neoplastic cells is a nonselective process that does not lead to the emergence of variants resistant to the cytolytic process or having altered metastatic properties.

MATERIALS AND METHODS

Animals. Specific pathogen-free adult C3H/HeN (mammary tumor virus negative) and C57BL/6 mice, 6 to 8 weeks old, were purchased from the Frederick Cancer Research Facility's animal production area.

Media and Reagents. Eagle's minimal essential medium was purchased from M. A. Bioproducts (Walkersville, MD). The medium was supplemented with 5% heat-inactivated fetal bovine serum, sodium pyruvate, sodium bicarbonate, nonessential amino acids, L-glutamine, and 50 μg gentamicin/ml (designated CMEM). HBSS without Ca2+ and Mg2+ was purchased from Grand Island Biological Co. (Grand Island, NY). MTP-PE was the kind gift of Ciba-Geigy, Ltd. (Basel, Switzerland). Egg phosphatidylycholine and bovine brain phosphatidylinerine were purchased from Avanti Biochemicals (Birmingham, AL).

Tumors. Tumors syngeneic to the C3H/HeN mouse were the UV-induced highly antigenic and regressor fibrosarcomas UV-2240, UV-1591, and UV-1316 (21); the immunogenic but progresser fibrosarcoma UV-2237, and cloned line 22 isolated from the progresser tumor UV-1422; a rhabdomyosarcoma, SR-149, that arose spontaneously in an aged C3H/HeN mouse in our colony; and a melanoma, K-1735, induced by a course of UV light exposures followed by chronic skin painting with croton oil (22). A 3H-induced osteogenic sarcoma, OS-J, syngeneic to the C57BL/6 mouse, was the kind gift of Dr. A. E. Reif, Boston City Hospital, Boston, MA.

1 Supported in part by a fund from the Kleberg Foundation.
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: NK, natural killer; MLV, multilamellar vesicles; MTP-PE, muramyl tripeptide phosphatidylethanolamine; NK, natural killer; PEM, peritoneal exudate macrophages; CMEM, complete minimal essential medium; HBSS, Hank’s balanced salt solution; IdUrd, idodeoxyuridine; mac-1, macrophage cycle variant 1 (other variants are similarly designated).
that had been activated with MLV-entrapped MTP-PE 24 hr previously. (104) were added to 38-sq mm wells containing macrophage monolayers trypsin and 0.02% EDTA), and resuspended in CMEM. Viable tumor cells (0.2 /iCi/ml; specific activity, 2000 mCi/mmol; New England Nuclear, unbound radiolabel, harvested by a 1-min trypsinization (0.25% Difco phase were incubated for 24 hr in CMEM supplemented with [125I]ldUrd simultaneously and grown in culture. Target cells in exponential growth and other aliquots were plated onto fresh cultures of activated macrophages for the next interactive selection cycle. This process was repeated 6 times, yielding variants mac-1 to mac-6 for each of the 8 phages for the Activation of Macrophages. Pure phosphatidylcholine and phosphatidylserine were admixed at a 7:3 molar ratio and dissolved in chloroform. MTP-PE dissolved in methanochloroform (1:2) was added to the phospholipids. After evaporation of the solvents and appropriate drying procedures, calcium- and magnesium-free HBSS was added, and MLV were produced by mechanical agitation. The concentration of MTP-PE in the MLV was 4 µg/µmol phospholipids, an optimum level to activate tumoricidal properties in murine macrophages (13).

Collection and Cultivation of Peritoneal Exudate Macrophages. PEC were collected by peritoneal lavage from mice given i.p. injections of 2 ml thioglycollate broth (Baltimore Biological Laboratories, Cockeysville, MD) 4 days before harvest (26). The PEC were centrifuged at 250 X g for 10 min, resuspended in serum-free medium, and plated either into Falcon No. 3042 Microtest II plastic tissue culture dishes (10^2 PEM/ well of 38-sq mm surface area) or Costar 3506 6-well tissue culture clusters (10^2 PEM/well of 962 sq mm surface area). After incubation for 40 min at 37°, the wells were rinsed with HBSS to remove nonadhering cells and processed as described below. The resultant monolayers of macrophages were >98% pure according to morphological and phagocytic criteria (26).

Sequential Interaction of Tumor Cell Lines with Activated Macrophages. Forty min after initial plating of PEM into 962-sq mm wells, the cultures were washed, and medium containing serum and MLV-entrapped MTP-PE (0.8 µg MTP-PE entrapped within 200 nmol phospholipid/well) was added to each well. Following a 24-hr activation period, the PEM were washed 3 times with medium to remove unphagocytosed liposomes, and 1 x 10^5 tumor cells were plated on the macrophage monolayers. Following a 96-hr coculture of macrophages and tumor cells, the cultures were washed twice with medium to remove nonadherent tumor cells. The remaining adherent viable cells were recovered by trypsinization of the monolayer for 2 min (0.25% trypsin:0.02% EDTA). In general, this procedure did not detach macrophages from the culture substrate, and those few macrophages that were detached did not replate. Thus, the viable tumor cells, designated macrophage cycle variant 1 (mac-1), were replated into fresh culture flasks and allowed to proliferate in the absence of macrophages for 7 to 17 days, depending on the tumor system. Aliquots of these cultures were cryopreserved, and other aliquots were plated onto fresh cultures of activated macrophages for the next interactive selection cycle. This process was repeated 6 times, yielding variants mac-1 to mac-6 for each of the 8 tumors.

To ascertain that at each cycle the PEM were indeed tumor cytotoxic, parallel quantitative assays of macrophage-mediated cytotoxicity, as described below, were carried out for each of the tumor targets.

Quantitative Assay of Macrophage-mediated Cytotoxicity. Macrophage-mediated cytotoxicity was assessed by a radioactive release assay described previously (26). All cryopreserved parent (starting) tumor lines and their respective mac-1 to mac-6 sublines were recovered simultaneously and grown in culture. Target cells in exponential growth phase were incubated for 24 hr in CMEM supplemented with [125I]ldUrd (0.2 µCi/ml; specific activity, 2000 µCi/mmol; New England Nuclear, Boston, MA). The cells were then washed 3 times with HBSS to remove unbound radiolabel, harvested by a 1-min trypsinization (0.25% Difco trypsin and 0.02% EDTA), and resuspended in CMEM. Viable tumor cells (10^7) were added to 38-sq mm wells containing macrophage monolayers that had been activated with MLV-entrapped MTP-PE 24 hr previously.

At this population density, normal (untreated) macrophages or macrophages incubated in MLV alone are not cytotoxic to tumor cells (26). No significant differences were detected in the plating efficiency of [125I]-ldUrd-labeled target cells to plastic or to control or liposome-treated macrophage populations. Radiolabeled target cells were also plated alone as an additional control group. The macrophage-target cell cultures were washed 24 hr after the initial plating of the target cells to remove nonplated target cells and then refed with CMEM. After an additional 48 hr of incubation, the cultures were washed twice with HBSS to remove nonadherent cells, and the remaining viable, adherent cells were lysed with 0.1 ml 0.1 N NaOH. The lysate was absorbed in a cotton swab and placed directly into 10- x 75-mm tubes. Radioactivity was monitored in a gamma counter. In this assay, maximal in vitro macrophage-mediated cytotoxicity is obtained after 3 days of macrophage-target cell incubation (26). The cytotoxic activity of the macrophages was calculated as follows:

\[
\text{% of cytotoxicity} = \frac{\text{cpm in target cells cultured with normal macrophages} - \text{cpm in target cells cultured with activated macrophages}}{\text{cpm in target cells cultured with normal macrophages}} \times 100
\]

Experimental results were analyzed for their statistical significance by Student’s 2-tailed t test.

Determination of Macrophage Content of Tumors. Animals were given injections s.c. in the inguinal region with viable tumor cells harvested from cultures. Tumors were removed en bloc at 1 to 1.3 cm in diameter, the nontumorous tissue was removed, and the tumors were weighed. Tumor fragments of approximately 1 cu mm were digested in trypsinization flasks at 37° with continuous mixing by a stirring bar. The digestion medium consisted of HBSS containing 0.14% collagenase type I and 0.03% DNase type 1 (Sigma, St. Louis, MO) (30).

The suspension of single cells obtained from the enzymatically dissociated tumors was adjusted to contain 5 x 10^6 cells in 1 ml of serum-free medium. A 0.1.ml suspension of India ink (1:40 dilution) was added to the cells. This mixture was placed into a 5-ml polystyrene tube and incubated for 2 hr at 37°. The cells were then washed twice with HBSS and resuspended in 5 ml (1 x 10^7 cells/ml) of medium containing 10% fetal bovine serum. Preparations of cells were fixed, stained with hematoxylin-eosin, and prepared for microscopy (30). The percentage of macrophages present in the preparations was determined by morphological criteria and ability to phagocytose carbon particles. A minimum of 200 cells/slide was examined to derive the percentage of macrophages.

Experimental Metastasis Production. Tumor cells were harvested from mid-log-phase cultures by a 1-min trypsinization as described above and washed twice in HBSS. Viability was assessed by trypan blue dye exclusion, and only suspensions with single cells of >95% viability were used. The tumor cell suspensions were adjusted to the required inoculum dosage in HBSS and injected into the lateral tail vein in a volume of 0.2 ml. Inocula were as follows: OS-J parent and OS-J-mac-5, 2 x 10^6 cells/mouse; K-1735 parent and K-1735-mac-6, 1 x 10^6 cells/mouse; and UV-2237 parent and UV-2237-mac-6, 1 x 10^6 cells/mouse. The mice were killed 28 days after the i.v. injection. Lungs were removed, rinsed in water, and fixed in Bouin’s fixative to outline tumor foci as opalescent white colonies. The pulmonary metastases were counted under a dissecting microscope.

Spontaneous Metastasis Formation. Tumor cells growing in culture were harvested as described above. The posterior footpads of mice were injected with 0.05 ml of tumor cell suspension containing the required tumor inoculum. Inocula were as follows: OS-J parent and OS-J-mac-5, 5 x 10^6 cells/mouse; K-1735 parent and K-1735-mac-6, 1 x 10^6 cells/mouse; and UV-2237 parent and UV-2237-mac-6, 1 x 10^6 cells/mouse. When the s.c. tumors reached a predetermined size (1 cm in diameter), the mice were anesthetized, and the legs with the tumors were amputated at the midfemur. The mice recovered and, 6 weeks...
gested that certain tumor cells can elaborate a factor that inhibits first infiltrate into growing tumors. Several studies have sug
achieve optimum interaction of macrophages and tumor cells. Due to a failure to activate PEM at any selection cycle. We base
macrophage migration into the tumor (28). Under such circum
here measure the ability of activated macrophages to lyse tu-
mediated lysis in vitro (Table 1). The in vitro assays described
select tumor cells with increased resistance to macrophage-
exhibit cytotoxicity against the tumor targets (data not shown). The susceptibility of all the tumor lines obtained from successive interactions with activated PEM (mac-1 to mac-6) to macrophage-mediated lysis did not significantly differ from their parental tumor lines. In 3 of 48 total macrophage-tumor cell interactions (UV-2237 mac-2, UV-1591 mac-2, and UV-2240 mac-1), a measurable decrease in sensitivity occurred. However, subsequent cycles of selection ended this trend. Our failure to select macrophage-resistant tumor cells was not due to a failure to activate PEM at any selection cycle. We base this conclusion on the data shown in Table 2, which demonstrated that, in each cycle, the PEM were indeed tumoricidal. Macrophage Content of the K-1735 Melanoma, UV-2237 Fibrosarcoma, and Their mac-6 Sublines Growing s.c. In the above in vitro studies, culture conditions were manipulated to achieve optimum interaction of macrophages and tumor cells. Under these conditions, the results demonstrate a failure to select tumor cells with increased resistance to macrophage-mediated lysis in vitro (Table 1). The in vitro assays described here measure the ability of activated macrophages to lyse tumorigenic cells. To do so in vivo, activated macrophages must first infiltrate into growing tumors. Several studies have suggested that certain tumor cells can elaborate a factor that inhibits macrophage migration into the tumor (28). Under such circumstances, macrophage-tumor cell interaction may not take place, allowing for tumor cells to escape from destruction. We therefore determined if the 6 consecutive interactions of tumor cells with activated macrophages could have inadvertently selected for tumors with decreased host-infiltrating macrophages. C3H/HeN mice were inoculated s.c. with the K-1735 melanoma, UV-2237 fibrosarcoma, and their mac-6 sublines. The induction time of tumors did not differ between parent lines and their mac-6 sublines (data not shown). Four weeks posttransplantation, the animals were killed, and tumors measuring 1 to 1.5 cm in diameter were resected and dissociated enzymatically. Infiltrative macrophages of the tumors were identified by macrophage morphology and phagocytosis of carbon particles (30). There were no demonstrable variations in the macrophage content of the parental tumors and their respective mac-6 lines (Table 3). In good agreement with our previous studies (30), macrophage content of the parent U-2237 fibrosarcoma was 12% of total cells. This was compared with an average macrophage content of 12% for the UV-2237 mac-6 variant. Similarly, the macrophage content of the parental K-1735 melanoma and K-1735 mac-6 subline were very similar, i.e., 11.6 and 13.3%, respectively.

**Experimental and Spontaneous Metastasis in Normal Syngeneic Recipients Produced by Parental Tumors and Sublines Surviving Successive Interactions with Activated Macrophages.** In the next set of experiments, we examined whether multiple interactions with tumoricidal macrophages could alter the metastatic potential of surviving tumor cells. This question was investigated by examining the experimental and spontaneous metastatic potential of the following tumors: the low metastatic OS-J parent osteogenic sarcoma and its mac-5 line; the highly metastatic K-1735 parent melanoma and its mac-6 line; and the metastatic UV-2237 parent fibrosarcoma and its mac-6 line. The induced metastasis produced by cells from these 6 test groups is shown in Table 4. The number of lung colonies produced by parental tumors and by cells surviving interactions with tumoricidal macrophages were very similar. The mac-5 subline of the low metastatic OS-J osteogenic sarcoma maintained low lung colony formation potential. The mac-6 sublines of the highly metastatic K-1735 melanoma and UV-2237 fibrosarcoma were also highly metastatic following i.v. injection.

Spontaneous metastasis of the 3 parent tumors and their

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**Table 1**

<table>
<thead>
<tr>
<th>Source of tumor cells</th>
<th>% of macrophage-mediated lysis against UV-2327</th>
<th>UV-1316</th>
<th>UV-1591</th>
<th>UV-2240</th>
<th>UV-1422 clone 22</th>
<th>Osteo-J</th>
<th>K-1735</th>
<th>SR-149</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental tumor</td>
<td>58</td>
<td>63</td>
<td>63</td>
<td>58</td>
<td>87</td>
<td>62</td>
<td>87</td>
<td>70</td>
</tr>
<tr>
<td>mac-1</td>
<td>85</td>
<td>84</td>
<td>74</td>
<td>58</td>
<td>66</td>
<td>68</td>
<td>88</td>
<td>81</td>
</tr>
<tr>
<td>mac-2</td>
<td>58</td>
<td>54</td>
<td>54</td>
<td>50</td>
<td>62</td>
<td>61</td>
<td>87</td>
<td>80</td>
</tr>
<tr>
<td>mac-3</td>
<td>84</td>
<td>70</td>
<td>63</td>
<td>65</td>
<td>65</td>
<td>60</td>
<td>74</td>
<td>80</td>
</tr>
<tr>
<td>mac-4</td>
<td>87</td>
<td>76</td>
<td>53</td>
<td>87</td>
<td>73</td>
<td>87</td>
<td>75</td>
<td>84</td>
</tr>
<tr>
<td>mac-5</td>
<td>83</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>50</td>
<td>58</td>
<td>87</td>
<td>75</td>
</tr>
<tr>
<td>mac-6</td>
<td>75</td>
<td>64</td>
<td>59</td>
<td>59</td>
<td>73</td>
<td>59</td>
<td>73</td>
<td>87</td>
</tr>
</tbody>
</table>

*a* Ten thousand tumor cells were plated onto PEM monolayers activated 24 hr before with 200 nmol MLV containing 0.8 μg MTP-PE. Cultures were incubated for 96 hr, and the remaining viable tumor cells were recovered by trypsinization of the monolayer. Tumor cells were expanded in the absence of macrophages and replated onto fresh cultures of activated PEM for the next cycle. This process was repeated 6 times.

*b* Ten thousand target cells labeled with [3H]TdR were plated onto PEM cultures treated 24 hr before as described in "Materials and Methods." Cultures were assayed 24 hr after addition of target cells, and cell-associated radioactivity was measured after 72 hr. The results are mean percentages of cytotoxicity from triplicate cultures.

*c* Five selections only.

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Table 2

Evidence for tumoricidal activation of macrophages used in various selection cycles

<table>
<thead>
<tr>
<th>Tumor target (parental)</th>
<th>% of specific macrophage-mediated lysis† with selection cycle:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>K-1735 melanoma</td>
<td>47</td>
</tr>
<tr>
<td>UV-2237 fibrosarcoma</td>
<td>45</td>
</tr>
<tr>
<td>149 rhabdomyosarcoma</td>
<td>65</td>
</tr>
<tr>
<td>OS-J osteosarcoma</td>
<td>73</td>
</tr>
</tbody>
</table>

*PEM (10^6) were activated for 24 hr with MLV-MTP-PE. 10^6 [125I]ldUrd-labeled target cells were added for 72 hr. Percentage of specific cytoxicity was calculated by comparison with noncytotoxic, control macrophages (p < 0.001).

†Five selections only.

Table 3

Macrophage content of s.c. growing neoplasms

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>Weight (g)</th>
<th>% macrophage content</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-1735 parent</td>
<td>1.4 (1.0-1.9)</td>
<td>11.6 (11-13)</td>
</tr>
<tr>
<td>K-1735, mac-6</td>
<td>1.8 (1.4-2.2)</td>
<td>13.3 (12-15)</td>
</tr>
<tr>
<td>UV-2237, parental</td>
<td>1.8 (1.2-2.4)</td>
<td>12.0 (11-13)</td>
</tr>
<tr>
<td>UV-2237, mac-6</td>
<td>1.4 (1.2-1.6)</td>
<td>12.0 (10-14)</td>
</tr>
</tbody>
</table>

‡ Obtained as described in Table 1. Footnote a. Ten mice per group were given injections s.c. with 2 x 10^3 single viable cells. Mice were killed 27 days later.

‡‡ Average weight of each tumor (n = 10).

‡§ Average of 10 tumors.

‡ Numbers in parentheses, range.

Table 4

Experimental metastatic potential of parent tumor cells and cells surviving 6 sequential interactions with macrophages

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>No. of lung colonies/mouse</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.S-J, parental</td>
<td>0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0</td>
<td>2 (0-4)</td>
</tr>
<tr>
<td>O.S-J, mac-5</td>
<td>0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0</td>
<td>3 (0-4)</td>
</tr>
<tr>
<td>K-1735, parental</td>
<td>24, 39, 58, 76, 87, 94, 114, 118</td>
<td>77 (24-118)</td>
</tr>
<tr>
<td>K-1735, mac-6</td>
<td>54, 63, 71, 82, 92, 105, 105, 110</td>
<td>87 (54-110)</td>
</tr>
<tr>
<td>UV-2237, parental</td>
<td>64, 163, 195, 197, &gt;300, &gt;300, &gt;300, &gt;300</td>
<td>197 (64-300)</td>
</tr>
<tr>
<td>UV-2237, mac-6</td>
<td>27, 121, 135, 168, 184, &gt;300, &gt;300, &gt;300, &gt;300, &gt;300, &gt;300</td>
<td>176 (27-300)</td>
</tr>
</tbody>
</table>

‡‡ Obtained as described in Table 1. Footnote a. Mouse were killed 28 days after i.v. injection. The number of pulmonary metastases was determined with a dissecting microscope.

‡§‡ Numbers in parentheses, range.

Table 5

Production of spontaneous metastases by parent tumor cells and cells surviving 6 sequential interactions with macrophages

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>No. of metastases</th>
<th>Primary tumor size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.S-J, parental</td>
<td>2/10</td>
<td>0 (0-2)</td>
</tr>
<tr>
<td>O.S-J, mac-5</td>
<td>3/10</td>
<td>0 (0-4)</td>
</tr>
<tr>
<td>K-1735, parental</td>
<td>9/10</td>
<td>21.5 (0-46)</td>
</tr>
<tr>
<td>K-1735, mac-6</td>
<td>8/10</td>
<td>22 (0-54)</td>
</tr>
<tr>
<td>UV-2237, parental</td>
<td>8/10</td>
<td>16.5 (0-33)</td>
</tr>
<tr>
<td>UV-2237, mac-6</td>
<td>9/10</td>
<td>21.5 (0-39)</td>
</tr>
</tbody>
</table>

‡ Obtained as described in Table 1. Footnote a.

‡‡ Mice were killed 6 weeks (K-1735 and UV-2237) or 10 weeks (O.S-J) following amputation of the primary tumor, and the number of pulmonary metastases was determined with a dissecting microscope.

‡§ Median number of metastases.

‡¶ Numbers in parentheses, range.

‡* Average ± S.D. at time of amputation.

variant lines was determined subsequent to s.c. implantation. The data are shown in Table 5. Here again, cells surviving multiple interactions with macrophages did not exhibit altered potential for spontaneous metastasis. The O.S-J parental and mac-5 cells were not metastatic, whereas the mac-6 sublines of the K-1735 melanoma or UV-2237 fibrosarcoma were as metastatic as were their parental tumors (Table 5).

DISCUSSION

After 6 sequential interactions of 8 different tumors with cytotoxic macrophages, we could not isolate a tumor variant with an increased resistance to macrophage-mediated lysis. Neither the antigenic property of the tumors nor their heterogeneous composition influenced this outcome. Moreover, cells surviving 6 sequential interactions with highly cytotoxic macrophages retained the metastatic potential of their parental neoplasms. The technique we used in attempting to select a macrophage-resistant cell was very similar to those used previously to successfully select tumor cells resistant to T-lymphocytes (7) or NK cells (15). The selection pressure used to isolate tumor cells resistant to T-cell or NK cell-mediated lysis was less severe than that used in the present study. Nonetheless, in contrast to macrophage-mediated lysis, T-lymphocyte or NK-cell mediated lysis is selective, and tumor cells resistant to such effector cells exhibit altered biological properties (7, 15). The present data and those published previously (4) suggest that all tumor cells are susceptible to macrophage-mediated lysis.

Much of our knowledge regarding the nature of tumor cell susceptibility to destruction by activated macrophages has come from studies quantitating release of intracellular radioisotopes (reviewed in Ref. 10). In the studies reported here, the sensitivity of tumor cells to cytotoxic macrophages was also assessed by the release of radiolabeled nuclear breakdown products. This 72-hr assay may well be distinct from many 4- to 18-hr short-term assays, which measure release of cytoplasmic radioactivity. This distinction is not trivial, since previously published data have demonstrated that, by extending tumor cell-macrophage interaction from 24 to 48 or 72 hr, significant increase in the level of target lysis for some relatively insensitive tumor targets can be achieved (33). Recently, Urban and Schreiber were able to select a variant line with increased resistance against macrophage-mediated lysis (short-term, 16-hr assay) from the UV-1591 fibrosarcoma (32). Since we failed to detect any increased resistance in the UV-1591 mac-6 (Table 1) after a 72-hr interaction with tumoricidal macrophages, we exchanged cell lines with Urban and Schreiber. We indeed found that their variant line UV-1591 MRV-1 was resistant to a 16-hr interaction with macrophages in an assay measuring the release of 51Cr from the target cells, yet the same target cells were fully susceptible to destruction by tumoricidal macrophages following a 72-hr interaction in an assay measuring the release of [125I]ldUrd from target cell DNA.4 This discrepancy in relative resistance or susceptibility to macrophages depending upon the assay used to measure the event is not surprising. Once activated, macrophages can produce a large number of cytotoxic factors (reviewed in Ref. 1). A tumor cell resistant to one factor may be just as susceptible to another factor.

In 2 systems (K-1735 melanoma and UV-2237 fibrosarcoma), successive interactions with tumoricidal macrophages did not produce cells which, upon s.c. transplantation, gave rise to tumors with decreased macrophage content (Table 3). Therefore, it is unlikely that such tumors could escape destruction mediated by activated macrophages (27). Previous studies from our laboratory (18) and others (23) have shown that, in mice, the macrophage content of metastases is very comparable to that of the primary tumors. Moreover, macrophage content of tumors could
well be independent of the antigenic properties of the neoplasms (18). Our present study expands these findings, since it demonstrates no reduction in the ability of macrophages to infiltrate parental and mac-6 variant tumors.

Since we have been using macrophages for treatment of metastases, we wished to determine whether tumor cells that escaped such destruction could have altered metastatic properties. Such was not the case. The metastatic potential of tumor cells surviving macrophage-mediated lysis did not differ from that of their parental neoplasms.

At least in vitro, tumoricidal macrophages have been shown to lyse tumorigenic cells regardless of other characteristics such as immunogenicity, metastatic potential, and sensitivity to various anticancer drugs (4, 6, 9, 11, 14). The present study shows that tumor resistance to macrophage-mediated lysis, measured by release of a radiolabel from target cell DNA, was not detected and thus could be very rare. Collectively, this study suggests that macrophage-mediated lysis of tumor cells is nonselective and therefore has the potential to circumvent the problems of cancer cell heterogeneity and the emergence of treatment-resistant tumor cells.

REFERENCES


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