Tumor Cytokinetics in the Presence of Normal, Alloimmune, or Bacillus Calmette-Guérin-activated Host Cells Simultaneously Assayed in Vivo and in Vitro

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ABSTRACT

Rates of tumor cell loss, replication, and growth were determined simultaneously for P-815 mastocytoma cells growing in the presence of syngeneic DBA/2 resident or proteose peptone-elicited macrophages and under allogeneic C57BL/6 nonimmune conditions. Under alloimmune conditions, measured parameters differed in vitro and in vivo but conclusions were consistent in that alloimmune host cells were cytolytic and cytostatic in vitro but not in vivo despite equivalent or greater effector to target ratios, presence or absence of endotoxin in the tumor inoculum, or changes in the injection schedule of Bacillus Calmette-Guérin. Similarly, Bacillus Calmette-Guérin-activated macrophages were cytolytic and cytostatic in vitro but not in vivo when admixed with tumor cells prior to injection into the leg. This study is the first simultaneously conducted cytotoxic analysis of a common pool of labeled tumor cells growing in vitro and in vivo using randomly selected mice as donors of host effector cells or as recipients of tumor transplantation. It demonstrates that activated macrophages which are cytolytic and cytostatic in vitro for P-815 cells may not function analogously in vivo in controlling tumor growth.

INTRODUCTION

The tumoricidal potential of activated macrophages has been demonstrated convincingly in cell culture systems but it is uncertain if macrophages which are cytolytic and cytostatic in vitro function analogously in vivo to control tumor growth. Cytotoxic activity in vivo by activated macrophages has not been measured directly but has been inferred from the observations that intrallesional injection of BCG may cause tumor regression (1, 2) in proportion to macrophage content (3), admixing BCG or Corynebacterium parvum-elicited cells with tumor cells often fails to protect and may actually stimulate tumor growth (14, 18, 19). In addition, peritoneal macrophages obtained from animals with chronic infection or after injection of liposomes containing macrophage-activating agents protect against metastasis (9). In addition, peritoneal macrophages obtained from animals with chronic infection or after injection of BCG and other agents when tested in vitro are tumoricidal (6, 10-13). However, effective BCG or C. parvum therapy requires T-cells in addition to macrophages (14-17) and admixing macrophages with tumor cells often fails to protect and may actually stimulate tumor growth (14, 18, 19). In addition, macrophages in culture are removed from the biological milieu of the peritoneal cavity in which mediators and metabolites are freely diffusible and in which the state and number of host effector cells are determined by the full complexity of the tumor-host interaction.

At the present time, there is no reliable evidence that important events in host resistance to tumors are measured by in vitro assays of macrophage effector function primarily because isotope release assays conducted at a single time point do not measure rates of tumor cytolysis and because isotope incorporation often used to document cytostasis does not measure rates of tumor proliferation. The single most important parameter in evaluating host effector function is overall tumor growth. Whereas existing assays failed to provide kinetic data relevant to tumor growth, we recently reported a method to simultaneously measure rates of tumor cell loss, replication, and growth applicable to in vitro analysis of macrophage-mediated cytotoxicity (20).

We now report results of cytotoxic experiments simultaneously conducted in vitro and in vivo using a common pool of P-815 mastocytoma cells and randomly selected mice either as donors of effector cells or as recipients of tumor transplants. The purpose of the first series of experiments was to validate the methodology developed in vitro for in vivo measurement by relating rates of tumor replication and loss to overall tumor growth in an alloimmune model wherein cytotoxicity was clearly present. The second series of experiments analyzed cytokinetic parameters of tumor growth in vitro and in vivo in the presence of BCG-activated macrophages.

MATERIALS AND METHODS

Tumor Cells. Cryopreserved murine P-815 mastocytoma cells were obtained from the Mason Research Institute, Worcester, MA, and passage in syngeneic DBA/2 mice by serial i.p. inoculation of 2 × 10^6 tumor cells. Tumor cells were harvested 3–5 days later by washing the peritoneal cavity with RPMI 1640 containing 10 units of heparin/ml. Yield of tumor cells averaged 200 million with greater than 95% viability by trypan blue dye exclusion. Tumor cells were either unlabeled or after labeling with [125I]ltdUrd (specific activity, 5 Ci/mm; Amersham Corp., Arlington Heights, IL). Cells were labeled in vivo by injecting i.p. 20 μCiIdUrd/mouse 16 h before tumor cell harvest. The amount of incorporated radioactivity did not exceed 12,000 cpm/10^6 cells. Previous studies have shown that this amount of IdUrd incorporation into P-815 cells does not alter P-815 replication (20). Tumor cells were tested periodically for the absence of mycoplasma and lactate dehydrogenase virus. DBA/2 mice and allogeneic C57BL/6 mice were obtained from The Jackson Laboratories, Bar Harbor, ME.

Macrophages. Macrophages were elicited in DBA/2 or C57BL/6 mice by i.p. injection of 1.3 ml of proteose peptone (Difco Laboratories, Detroit, MI). Macrophages were activated by i.p. injection of viable BCG (Tice strain) at an approximate concentration of 10^9 organisms/mouse. Except where indicated, BCG was injected 17 days and proteose peptone 3 days before experimentation. This time period was selected since others have shown that the optimal time for obtaining activated macrophages is 2 to 3 weeks after BCG injection (21). Simultaneous experiments were performed by i.p. inoculation of tumor cells (2 × 10^6) and by culture of tumor cells in the presence of harvested peritoneal effector cells. Effector cells for in vitro assay were eluted from the peritoneal cavity of mice with 10 ml of medium, washed three times, and resuspended at a final concentration of 1 × 10^6 macrophages/ml. The in vivo E/T ratio was estimated by the ratio of macrophages to tumor cells harvested from the peritoneal cavity 1.5 h after tumor cell inoculation. Lipopolysaccharide was obtained from Sigma Chemical Co., St. Louis, MO, and was prepared from Escherichia coli by phenol extraction.

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2The abbreviations used are: BCG, Bacillus Calmette-Guérin; LPS, lipopolysaccharide; E/T, effector to target ratio; IdUrd, iododeoxyuridine.

2067
TUMOR CYTOKINETICS

Cell Culture. Culture medium consisted of RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum and 1% antibiotic-antimyotic mixture containing penicillin (10,000 units/ml), streptomycin (10,000 μg/ml) and Fungizone (25 μg/ml). Costar plastic tissue culture plates of 16-mm diameter and 18-mm depth were obtained from Rochester Scientific Co., Rochester, IL. Cells were incubated in an atmosphere of 5% CO₂ and 95% relative humidity. When tumor cells were cultured in the presence of peritoneal cells, suspension cultures were used without selection by adherence. Except where indicated, E/T ratios were based on macrophage content. They were generally 1:1 consisting of 1 ml each of peritoneal cells (1 x 10⁶ macrophages/ml) and tumor cells (1 x 10⁶/ml).

Measurement of Tumor Cell Loss. Aliquots from a common pool of [¹²⁵I]labeled tumor cells were injected i.p. or placed in culture. At selected intervals, incorporated radioactivity was measured as cpm using a Beckman Gamma 300 radiation counter for cells harvested from both the peritoneal cavity and culture wells. Whole body counts were made on mice placed in a specially designed carrier adapted for use with the gamma counter. The rate of tumor cell loss was computed as the slope of the regression line fitted between In cpm versus time. Data were obtained at four intervals with the exact times recorded. The time periods selected were based on expected rates of cytotoxicity and are indicated for each experiment.

Measurement of Tumor Cell Growth. Total cell counts were made for each harvest from both the peritoneal cavity and culture wells using a Coulter Counter model ZF (Coulter Electronics Inc., Hialeah, FL) or hemacytometer, respectively. Differential cell counts were performed on cytocentrifuge slides stained with Wright's Giemsa and used to correct total cell counts to total tumor cells. The rate of tumor growth was calculated as the slope of the line fitted between In cell cpm versus time. In an exponentially growing population, the population doubling time can be calculated as 2ⁿ/r (22).

Measurement of Tumor Cell Replication. For isotopically labeled cells, tumor replication was measured by the rate of change in isotope specific activity with time (20). For each cell harvest, specific activity was calculated by dividing cell cpm by total tumor cell number and the rate of tumor replication computed as the slope of the regression line fitted between ln specific activity versus time. The replicative rate for unlabeled tumor cells in vivo was based upon metaphase arrest using vincristine sulfate (Sigma Chemical Co., St. Louis, MO). First, the optimal concentration of vincristine to arrest P-815 cells in mitosis was determined and found to be 1 μg/g body weight. Second, this concentration of vincristine was administered 18 h after tumor transplantation. At 1, 2, 4, and 6 h later, peritoneal cells were harvested and slides prepared using the cytocentrifuge. Slides were stained with Wright's Giemsa and the mitotic index determined from a 500-cell count as the percentage of cells in mitosis. The replicative rate was determined as the slope of the line fitted between mitotic index and time (22).

Cell Irradiation. P-815 mastocytoma cells (8 x 10⁶ cells/ml culture medium) were irradiated with 6000 rads from a cesium source (model 225 S to 182 S, Nuclear Enterprises, Inc., Middlesex, England). Freeze Thawing. Six ml of cell suspension containing 1 x 10⁶ cells/ml were three times frozen in an acetone-dry ice mixture and thawed at 37°C. At selected intervals, incorporated radioactivity was measured as cpm using a Beckman Gamma 300 radiation counter for cells harvested from both the peritoneal cavity and culture wells. Whole body counts were made on mice placed in a specially designed carrier adapted for use with the gamma counter. The rate of tumor cell loss was computed as the slope of the regression line fitted between In cpm versus time. Data were obtained at four intervals with the exact times recorded. The time periods selected were based on expected rates of cytotoxicity and are indicated for each experiment.

RESULTS

Cytokinetics of P-815 Cells in Culture and in Syngeneic Normal Mice. P-815 mastocytoma cells from a common pool of isotopically labeled [¹²⁵I]labeled tumor cells were seeded into culture or transplanted i.p. to syngeneic normal mice and their rates of cell loss, replication, and growth measured over the ensuing 72-h period. One group of mice was used for whole body counts while a separate group was sacrificed at intervals for peritoneal cell harvest. The data are presented in Table 1. There was no significant difference in the rate of tumor cell loss calculated from whole body counts compared to cells harvested from the peritoneal cavity and the latter did not differ from cells harvested from culture wells. Further, the rate of tumor replication as measured by the rate of change in ln specific activity with time showed no significant difference between in vitro and in vivo conditions. Similarly, the measured rate of tumor growth was the same for the two conditions. The cytokinetic parameters obtained from harvested peritoneal cells were considered accurate for in vitro growth of P-815 tumor cells because of concordance with in vitro data as well as whole body counts and because the rate of tumor growth (k₂), the rate of tumor replication (k₁), and the rate of tumor cell loss (k₃) were related as follows (20, 21):

\[ k₂ = k₁ - k₃ \] (B)

Isotopically labeled tumor cells were compared to unlabeled cells after injection into syngeneic normal mice (Table 2). The rate of tumor replication for unlabeled cells was measured using metaphase arrest with an optimal concentration of vincristine sulfate (1 μg/g body weight). The replication rate of these unlabeled tumor cells was not significantly different from that calculated for labeled tumor cells using the rate of change in isotope specific activity. Based upon harvested cells, the calculated rate of tumor growth was also not significantly different between unlabeled and labeled cells. Using Equation B, the calculated rate of tumor cell loss for unlabeled cells was 0.024 cells/h which was identical to the 0.025 cells/h measured for labeled cells. Based on this data, the labeled cells behaved similarly to unlabeled cells.

Tumor cells were irradiated with 6000 rads to inhibit cell division while retaining viability. The rate of cell loss for these cells was identical to nonirradiated tumor cells although their rate of replication was markedly reduced (Table 2). Further, the

<table>
<thead>
<tr>
<th>Determination</th>
<th>In vivo</th>
<th>In vitro</th>
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<tbody>
<tr>
<td>Whole body</td>
<td>Harvested cells</td>
<td>Suspension culture</td>
</tr>
<tr>
<td>Rate of tumor cell loss</td>
<td>0.021 ± 0.004</td>
<td>0.018 ± 0.003</td>
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<tr>
<td>Half-life (h)</td>
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<td>38.5</td>
</tr>
<tr>
<td>Rate of tumor replication</td>
<td>0.080 ± 0.006</td>
<td>0.069 ± 0.005</td>
</tr>
<tr>
<td>Rate of tumor growth</td>
<td>0.058 ± 0.004</td>
<td>0.047 ± 0.003</td>
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*NA, not applicable.
growth rate of irradiated tumor cells had a negative slope indicating tumor regression at a magnitude equal to the difference in the rates of tumor replication and loss. This data confirmed the use of the slope of the In specific activity versus whole body counts over a 24-h period. The rate of isotope retention in tumor cells were transplanted to allogeneic C57BL/6 mice or cultured of tumor metastases. This experiment suggested that whole gastrointestinal tract (6%), head and neck (10%), and carcass (11%) were the major sites of retained isotope. This isotope was not precipitated by cold trichloroacetic acid or extractable by hot trichloroacetic acid indicating that it was not contained in nuclei of tumor metastases. This experiment suggested that whole body counts might err in estimating rapid tumor lysis due to retained cpm and that rates based on harvested cells were preferred for quantifying cytolysis.

Tumor Cytokinetics under Allogeneic Conditions. The purpose of this experiment was to validate the sensitivity of cytokinetics to detect host tumoricidal activity in vivo. P-815 mastocytoma cells were transplanted to allogeneic C57BL/6 mice or cultured in the presence of cells harvested from the peritoneal cavities of such mice. Both immune and nonimmune conditions were examined on the premise that immune mice should rapidly kill the tumor cells. Under nonimmune conditions, the rate of tumor cell loss based upon whole body counts was identical to that determined from harvested peritoneal cells and the half-life of tumor cells in vivo was slightly longer than that observed in vitro (Table 3). However, tumor cell replication was greater in vitro than in vivo so that overall tumor growth was identical in suspension cultures compared to peritoneal ascites tumor growth. It is interesting that under nonimmune allogeneic conditions, tumor cytokinetics in vivo were identical to those observed under syngeneic conditions (Table 1). When immune C57BL/6 mice were examined (Table 3), the rate of tumor cell loss was dramatically enhanced over nonimmune conditions. The rate of tumor cell loss based upon whole body counts did not agree with that obtained from harvested peritoneal cells (0.672). Similar to freeze-thawed cells, this situation reflected the time required for isotope excretion and the fact that some isotope was retained in body structures. In vitro, the immune state entirely suppressed tumor replication. Consequently, the high rate of tumor cell loss was directly translated into a large negative value for the overall rate of tumor growth. Thus the immune state in vivo caused tumor regression by being both cytostatic and cytolytic. In vitro, suspension cultures of immune compared to nonimmune peritoneal cells showed a more rapid rate of tumor cell loss (cytolysis), a decreased replication rate (cytostasis), and tumor regression. The E/T ratio for immune conditions was calculated based on total cells and in vivo was 17:1 whereas the suspension cultures had an E/T ratio of 10:1. This may have contributed to the longer half-life and higher replicative rate in vitro than in vivo. However, it was not expected that these values be identical but rather that conclusions derived from in vivo and in vitro experiments be concordant. This was in fact true as immune cells in vivo and in vitro were both cytolytic and cytostatic in degree sufficient to completely suppress overall tumor cell growth.

The results are presented in Table 5 and demonstrate that this modification had no effect on tumor cytokinetics. Similar results were obtained with BCG injected at the time of tumor inoculation but also over the duration of the clinical illness was verified by noting that the mean time to death in BCG-treated animals was the same as the control. In a separate experiment, the tumor inoculum was reduced to 5 x 10^4 cells (E/T ratio of 110:1) with results similar to that shown. Second, we considered the possibility that the timing of BCG administration was critical. Therefore, BCG was given alone at t-4 days, or as two injections at t-4 and t-17. The results are presented in Table 5 and demonstrate that this modification had no effect on tumor cytokinetics. Similar results were obtained with BCG injected at the time of tumor growth.
challenge. Third, we considered that BCG macrophages in vivo might exist in the primed state and require LPS to trigger tumoricidal activity (24). We selected a dose of LPS (100 ng/mouse) which is 10 times that normally given to activate primed macrophages in vitro and which represents a concentration of LPS that has been shown to elicit macrophages in vivo with enhanced cytolytic activity in vitro (25). However, the simultaneous administration of LPS with the tumor cells into animals that had received prior BCG treatment resulted in neither tumoricidal activity in the immediate period nor prolongation of life over the longer period. From this series of experiments, we concluded that tumoricidal activity of BCG macrophages could not be demonstrated in vivo in the peritoneal cavity despite the fact that such macrophages in culture were tumoricidal.

Passive Transfer Experiment. Tumoricidal activity of macrophages...
cytotoxicity by BCG-activated macrophages in vivo was due to the geometry of the peritoneal cavity impeding close association, then admixture of effector and target cells with subsequent inoculation into a confined space should result in cytolysis. Parallel experiments were performed in which a common pool of tumor cells labeled with [\(^{125}\)I]IodUrd were admixed in vitro with various effector cells each from a common pool and then placed either in culture or passively transferred to the leg. At various times thereafter, tumor cells in culture were harvested concurrently with leg amputation. Residual labeled tumor cells were counted in a gamma counter and the rate of tumor cell loss calculated over a 72-h period. When P-815 tumor cells alone were examined, the rate of tumor cell loss and consequently tumor half-life was the same in vivo and in vitro (Table 6). There was no significant change in the rate of tumor cell loss in the presence of peptone-elicited macrophages either in culture or in the leg. In the presence of BCG-activated, peptone-elicited macrophages, the rate of tumor cell loss was markedly increased in vitro but not in vivo. The half-life of the tumor cells in vitro was reduced from a control value of 29 h to 13 h but in vivo the values were 28 h control versus 26 h with BCG-activated macrophages. This failure to demonstrate tumoricidal activity in vivo was not due to technical problems associated with isotope loss from the leg following tumor cell death because rapid in vivo cytosis of tumor cells did occur in the presence of alloimmune peptone-elicited cells. These latter effector cells increased tumor cell loss equivalently in vivo and in vitro so that half-life was reduced to 8 h in both conditions.

**DISCUSSION**

It is generally assumed that in vitro tests of effector cell function accurately measure activities expressed in vivo. In the present experiments, this assumption was correct for nonactivated macrophages which were not tumoricidal and for alloimmune effector cells which were highly cytotoxic and cytostatic. However, this assumption was not confirmed for BCG-activated macrophages. Such effector cells were sufficiently cytostatic and cytolytic as to prevent tumor growth in vitro but neither activity was demonstrated in vivo.

Only a few studies have attempted to define antitumor activity of macrophages in vivo. Nearly all such studies measure tumor take or mean time to death. Because such measurements are not particularly sensitive to the early events that follow tumor transplantation, interpretation may be complicated by host-derived cells. In a recent study by Fernandez-Cruz et al. (27), prolongation of life was induced by passively transferred macrophages activated in vitro by interferon-rich lymphokines. Although highly tumoricidal in vitro, it is questionable if the passively transferred macrophages were tumoricidal in vivo since prolongation of life required host-derived radio-sensitive cells and noticeable regression of tumors did not occur until 10 days after transplantation. Our data are consistent with this interpretation since we were unable to document tumoricidal activity in vivo by BCG-activated macrophages during the critical time period immediately after transplantation when the activity of the prior-activated macrophages should be maximally expressed and independent of subsequently generated host responses.

Our studies are the first to measure concurrently cytokinetic parameters of tumor growth in vivo and in vitro using the same pool of tumor cells and mice selected at random as donors for cell culture or as transplant recipients. In addition, the same anatomical site from which effector cells were harvested (peritoneal cavity) was used also for tumor cell inoculation. We selected the P-815 mastocytoma for study because it grows well in culture, s.c. tissue, and in the peritoneal cavity and because it is a commonly used, sensitive target for activated macrophages that is not particularly susceptible to natural killer cell activity (28).

The following data support the contention that our cytokinetic assay accurately measured P-815 tumor behavior in vivo. First, there was general concordance between cytokinetic parameters in vivo and in vitro with the exception of BCG-activated macrophages. This agreement may have been fortuitous reflecting the long adaptation of these tumor cells to both culture and ascites growth. Second, cytostasis was evident in tumor cells whose division had been arrested by irradiation and cytotoxicity was clearly demonstrable under alloimmune conditions in vivo. Further, labeled and unlabeled cells behaved identically. Third, results obtained from the peritoneal cavity agreed with tumor cell survival data obtained by admixed cells passively transferred to the leg. However, the advantage of the peritoneal cavity was that harvested cells could be used to obtain data on tumor replication and overall growth. Fourth, individually determined parameters were consistent in that rates of replication and rates of loss accurately predicted the overall rate of tumor growth (Equation B).

Various parameters were evaluated seeking explanation for the failure of BCG-activated macrophages to exhibit cytotoxicity in vivo despite impressive activity in vitro. First, the tumor inoculum was reduced to as low as 50,000 cells thereby increasing the E/T ratio to as high as 110:1. Although this was the lowest inoculum size that could be monitored for cytokinetics, the results showed no evidence of tumoricidal activity. Second, varying the timing of BCG inoculation relative to tumor cell challenge as well as multiple BCG injections were unsuccessful in yielding tumoricidal macrophages in vivo. Third, on the premise that BCG might prime macrophages to a nontumori
cidal state, a second signal consisting of LPS was given mixed in the tumor inoculum (24, 29). However, LPS did not trigger tumoricidal activity in vivo. The reduction in harvested macrophages following LPS injection was expected and attributed to a "macrophage disappearance reaction" (30). Fourth, the geometry of tumor macrophage interaction was changed so as to bring the tumor cells into close contact with the macrophages.

**Table 6** Cytolysis of P-815 cells by admixed effector cells in vitro and following passive transfer to leg (Winn-type assay)

Rates are reported as ± SD from one experiment, which was representative of three experiments. For all experiments, the E/T ratio was 10:1 adjusted for 2 x 10\(^6\) P-815 cells. The rate of cell loss was computed as the slope of the regression line formed between CPM in harvested cells (in vitro) or in tumor bearing legs (in vivo) versus time.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Rate of tumor cell loss (cells/h)</th>
<th>Half-life (h) (cells/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>In vivo</td>
<td>In vitro</td>
</tr>
<tr>
<td>Tumor alone</td>
<td>0.024 ± 0.001</td>
<td>0.025 ± 0.003</td>
</tr>
<tr>
<td>Tumor + syngeneic peptone-elicited macrophages</td>
<td>0.033 ± 0.002</td>
<td>0.026 ± 0.004</td>
</tr>
<tr>
<td>Tumor + syngeneic BCG-activated, peptone-elicited macrophages</td>
<td>0.033 ± 0.004*</td>
<td>0.027 ± 0.003</td>
</tr>
<tr>
<td>Tumor + allogeneic immune, peptone elicited cells</td>
<td>0.091 ± 0.007*</td>
<td>0.088 ± 0.009*</td>
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* Statistically significant difference from P-815 alone.  

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by admixing them prior to s.c. injection. This assay also failed to demonstrate cytolytic of the tumor cells by macrophages which were concurrently cytolytic in vitro.

We cannot exclude the possibility that P-815 cells are not a susceptible target for activated macrophages in vivo and studies with other cell types may be warranted. However, our results clearly demonstrate a discordance in tumor cell susceptibility to activated macrophages in vivo compared to in vitro. Further, it is possible that tumoricidal activity in vivo in the presence of tumor cells is rapidly lost and that multiple injections of activating agents are necessary to sustain tumoricidal activity. Such might be achieved with liposome encapsulated activating agents as such procedures have been reported to induce macrophage tumoricidal activity in vivo (9).

Intralesional, usually intradermal, injection of BCG suppresses tumor growth of some but not all tumors and clinical trials with this agent have been disappointing. Peritoneal exudate cells from BCG-injected mice have been reported to have no effect (14) or to inhibit tumor growth when passively transferred with tumor cells (4-6). Although differential susceptibility of tumor cells to activated macrophages might cause conflicting results, prolongation of life with passively transferred activated macrophages may relate more to their capacity to induce effective host resistance in the recipient than to their actual tumoricidal activity. Effective BCG or C. parvum responses involve T-cells (1, 14-17) and adjuvant activity against tumor-associated antigens may account for some success with these agents (31, 32). Then also, prolongation of life with passively transferred lymphokine-activated macrophages was delayed and dependent upon radiosensitive host cells (27). Alternatively, BCG protection appears limited to about 10 times the tumorigenic dose (14). If so, protection against P-815 tumors might extend to no more than 1000 cells suggesting that the dose of tumor cells required for our experiments may have been too great to observe prolongation of life.

One reason we developed a kinetic assay for macrophage cytotoxicity was that commonly used isotope release assays are often misleading with respect to the actual amount of tumor killing (20) and because there is no quantitative way to correlate isotope release data to inhibition of tumor growth in vivo. We have now applied this assay to a correlative in vivo study and demonstrated concordance in assay results for allogeneic effector cells but not for BCG-activated macrophages. Understanding the reasons for these results may help explain why impressive tumoricidal activity is often observed in vitro but the tumor grows remarkably well in vivo and why observed reactivity in vitro frequently does not correlate with extent of disease or prognosis.

REFERENCES

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