Antitumor Activity of Bacterial Infection. I. Effect of Listeria monocytogenes on Growth of a Murine Fibrosarcoma

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SUMMARY—Growth of a murine fibrosarcoma was suppressed when tumor cells were mixed with viable Listeria monocytogenes (LM) before intradermal injection into nonimmune syngeneic recipients. Immunization of recipients, by intravenous injection of LM 11 days before transplantation of LM-tumor cell mixtures, eliminated the mortality associated with large doses of LM but did not alter the antitumor activity of the microorganisms. Simultaneous injection of LM and tumor cells at separate sites failed to affect tumor growth, which suggested that contact between LM and tumor cells was required for tumor suppression. Tumor-specific immunity was not observed; mice surviving injection of LM and tumor cells did not resist a second tumor-cell challenge. At least 100 times more heat-killed LM was required to produce the antitumor effect of viable organisms. The ability of heat-killed LM to suppress tumor growth was abolished by treatment of recipients with rabbit antiserum to mouse thymocytes, which was consistent with a requirement for a host response to the LM. Regression of established fibrosarcoma transplants was produced by the intratumor injection of viable LM 5 days after injection of tumor cells. Intratumor injection of BCG at this interval was not effective. The incidence of tumor regression was not increased by multiple intratumor injections of LM, by intratumor injection of a combination of LM and BCG, or by preimmunization with LM prior to the intratumor injection of the same organism.—J Natl Cancer Inst 54:749-756, 1975.

INHIBITION OF TUMOR GROWTH has been achieved in several experimental systems by treatment with various infectious agents and their products (1–12). To date, however, clinical investigations with BCG (1), Corynebacterium parvum (13, 14), C. granulosum (15), and Bordetella pertussis (16) have produced only equivocal evidence for the efficacy of these agents in treatment of human cancer. More effective clinical immunotherapy might be facilitated by additional animal studies to determine the basis for the immunotherapeutic effect of different microorganisms, to define optimal conditions for the administration of these unusual antitumor agents, and to compare the relative antitumor activity of different microbes in the same tumor system.

One property shared by several bacteria that possess immunotherapeutic activity is the ability to stimulate acquired cellular immunity in the infected host. BCG (1), Brucella abortus (7, 8), Salmonella (9), and Listeria monocytogenes (LM) (7) stimulate the host’s immune response and can inhibit tumor growth. In previous studies we demonstrated suppression of tumor growth in mice and guinea pigs at the site of infection with BCG (16, 17). Treatment with agents and procedures known to compromise the host’s ability to control mycobacterial infection abolished the antitumor activity of BCG in these species (18, 19).

Much is known about the development and expression of immunity to LM (20–22). Studies of the antitumor activity of LM may further clarify the relationship between acquired cellular immunity and the inhibition of tumor growth during infection. In this paper and in a subsequent report (23) we define the antitumor activity of LM in mice and guinea pigs, comparing the effects of LM to those of BCG.

MATERIALS AND METHODS

Animals.—Male C3H/HeNc mice, 18–20 g, were obtained from the breeding colony at Frederick Cancer Research Center, Frederick, Maryland. Animals were housed in groups of 6–10 in plastic cages and fed Wayne Lab Blox and tap water ad libitum.

Tumors.—Tumor 1038 was induced in 1967 by a pellet of 1% 3-methylcholanthrene in paraffin in the subcutaneous tissue of a C3H/HeNc male mouse (17). Transplant generations 40–54 were used in these experiments. The tumor was maintained by serial passage in immunodepressed, syngeneic mice as described in (17). Split-thickness skin grafts from C3H/-HeNc donors survived without contraction on normal C3H/HeNc recipients for more than 100 days (17, 19).

Tumor cell suspensions.—Tumors were excised from donor animals and placed in Dulbecco’s phosphate-buffered saline (PBS) without calcium, magnesium, or antibiotics. Connective tissue and areas of necrosis were trimmed and the remaining tumor was minced into pieces 1–2 mm in greatest dimension. The minced tumor was digested for 15 minutes with 0.25% pronase and 40 μg DNAase/ml in PBS. Digestion was repeated 3–5 times; the first batch was usually discarded as it contained many nonviable cells. Dispersed tumor cells were sedimented at 200 × g for 10 minutes at ambient temperature, washed once, and resuspended in Hanks’ balanced salt solution supplemented with 10% fetal calf serum (HBSS-FCS). A fraction of the tumor cell suspension was diluted 1:20 with 0.13% trypan blue. Unstained cells were considered viable and counted in a hemacytometer.

Organisms.—LM, strain EGD, was maintained in a virulent state by repeated passage in mice. Organisms isolated from the spleen of an infected mouse were grown for 16–18 hours in trypticase soy broth (TSB),...
frozen, and stored at -70°C until use. In regression experiments, LM organisms were subcultured once without passage in mice. For each experiment, we enumerated colony-forming units (CFU) of LM by diluting a portion of the inoculum in 0.9% NaCl and plating samples of each dilution in brain-heart infusion agar. Colonies were counted after 48 hours' incubation at 37°C. To prepare heat-killed LM, a suspension of viable organisms was incubated at 90°C for 60 minutes. After being heated, the bacteria were washed and lyophilized. Loss of viability was confirmed by plating of a saline suspension of lyophilized heat-killed organisms (10 mg/ml) on sheep blood agar and on brain-heart infusion agar.

BCG, Tice strain, was obtained from the American Type Culture Collection (ATCC). The mycobacteria were grown as a dispersed culture in Middlebrook's medium 7H9 with 0.2% Tween-80, frozen, shipped, and stored at -70°C as described in (24). To enumerate organisms, suspensions were diluted in 0.9% NaCl supplemented with 1% gelatin; aliquots of appropriate dilutions were plated on Dubos oleic acid-albumin agar. Colonies were counted after 2 weeks' incubation at 37°C.

**Results**

**Suppression of tumor growth.**--Line-1038 fibrosarcoma cells were diluted with HBSS-FCS. LM and BCG were diluted in the same medium. Mixtures were prepared immediately before injection. To inject the largest numbers of living LM, organisms in undiluted TSB were mixed with equal volumes of tumor cell suspension. For tumor growth controls, tumor-cell suspensions were mixed with an equal volume of either TSB or HBSS-FCS. All injections were made intradermally (id) in a volume of 0.05 or 0.1 ml with a 27-gauge needle. Mixtures of bacteria and tumor cells were injected into skin covering the posterior dorsal surface of mice. In some experiments, tumor cells were inoculated on the dorsal surface and LM on the ventral surface. Animals were challenged by id injection of line-1038 tumor cells on the ventral surface.

**Regression of established tumor transplants.**--Tumors were established by id injection of 5 × 10⁴ line-1038 cells in 0.05 ml HBSS-FCS. At different times thereafter, suspensions of LM or BCG were injected directly into the tumor site in 0.10 ml with a 27-gauge needle. Mixtures of bacteria and tumor cells were injected into skin covering the posterior dorsal surface of mice. In some experiments, tumor cells were inoculated on the dorsal surface and LM on the ventral surface. Animals were challenged by id injection of line-1038 tumor cells on the ventral surface.

**Immunization with LM.**--Mice were immunized to LM by intravenous (iv) injection of 10⁸ CFU in 0.1 ml 0.9% NaCl 11 days before the injection of tumor cells or of LM-tumor cell mixtures (25).

**Treatment with antithymocyte serum.**--Mice received intraperitoneal (ip) injections of 0.25 ml rabbit antiserum to mouse thymocytes (ATS; lot #13209; Microbiological Associates, Bethesda, Md.) on day 5, 3, and 1 before and on day 1, 3, and 5 after injection of tumor cells with or without heat-killed LM. One control group received ip injections of 0.25 ml normal rabbit serum (NRS) according to the same schedule. A second control group received no ip injections.

**Evaluation of tumor growth.**--Mice were observed weekly for signs of progressive tumor growth. Of the animals that received line-1038 cells without LM or BCG, 98% (378:387) survived at least 2 weeks. Mice that died within 2 weeks after injection of bacteria were considered to have succumbed to bacterial infection. Tumor incidence was calculated from the number of mice surviving at least 2 weeks. Animals were usually observed for 90 days; in some experiments, for up to 120 days. Tumor incidence did not change after 60 days, and regression of untreated palpable tumors was not observed. Differences among groups were evaluated statistically by the Fisher exact test for groups of less than 20 and chi-square analysis for groups of more than 20.

**Results**

**Suppression of Tumor Growth With Viable LM or BCG**

To determine the effect of local injection on the growth of murine fibrosarcoma transplants, nonimmune mice received id injections of one of the following cell mixtures: line-1038 tumor cells plus LM; line-1038 tumor cells plus BCG; or line-1038 tumor cells alone. The number of LM or BCG was varied by one-log increments in different groups. Growth of 5 × 10¹⁰ line-1038 cells could be suppressed completely by mixture with 1 × 10⁶ viable LM immediately before transplantation, and suppression of a significant number of tumor transplants was produced with as few as 10⁴ organisms (table 1, text-fig. 1). Within 2 weeks after id injection of 10⁵-10⁷ LM, 15-60% of mice died. Early death was not observed with doses of 10⁴ organisms or less. Local injection with 10⁷ BCG suppressed a fraction of line-1038 tumor transplants, whereas 10⁴ or 10⁵ BCG was not effective. When equal numbers of BCG and LM were compared, LM was consistently more effective in suppressing growth of this particular tumor line.

A tenfold increase in the size of the tumor cell inoculum did not affect the fraction of transplants that could be suppressed with 10⁵-10⁷ LM (table 1, text-fig. 1). Increase in the tumor cell inoculum appeared to increase the number of tumors that developed from tumor cells and BCG, but the differences were not statistically significant. With 5 × 10⁴ line-1038 tumor cells, there was still a highly significant difference (P<0.001) between the effect of LM and a comparable number of BCG organisms.

**Suppression of Tumor Growth With Heat-Killed LM**

Having observed that a number of mice died after id injection of 10⁵-10⁶ viable LM, we evaluated the antitumor activity of heat-killed organisms incapable of producing progressive infection. Mixtures containing 10⁵-10⁸ heat-killed LM and 5 × 10⁵ line-1038 tumor cells were injected id into nonimmune mice. Statistically significant suppression of tumor growth was obtained with 10⁷ heat-killed organisms in each of two experiments (table 2). When the dose response obtained from the combined results of these two experiments was compared to that observed in earlier studies with viable LM, the two curves appeared similar, but = 100 times more heat-killed organisms...
were observed in mice receiving heat-killed LM. Injections of tumor cells with or without LM. There were required to produce an antitumor effect equal to that of viable LM (text-fig. 2). No early deaths were observed in mice receiving heat-killed LM.

Effect of Immunization With LM on Host Response to LM-Tumor Cell Mixtures

Mice given a sublethal dose of viable LM resist a second infection with numbers of organisms that would kill nonimmune animals (26). Consequently, immunization with LM provided another method for reducing the mortality associated with the injection of $10^5$-$10^7$ viable organisms. Mice received an iv inoculation of $10^6$ LM 11 days before the id injection of LM-tumor cell mixtures or tumor cells alone. Nonimmune mice that had not received iv injections of LM also received injections of tumor cells with or without LM. There was no consistent difference in the incidence or rate of tumor growth in LM-immune and nonimmune recipients, though $10^6$ LM organisms were significantly more effective in nonimmune hosts (table 3). Fewer deaths occurred in preimmune animals that received id injections of $10^5$ or $10^7$ LM in combination with tumor cells ($P=0.032$ and $P=0.003$, respectively).

Requirement for Contact Between LM and Tumor Cells for Suppression of Tumor Growth

Contact between BCG and tumor cells appeared to be essential for optimal BCG-mediated suppression of tumors in the rat (27), mouse (17), and guinea pig (16). To determine whether this was also true for the antitumor activity of LM, mice received LM and tumor cells at the same site or two different sites. Significant suppression of tumor growth was obtained when $10^6$-$10^7$ LM organisms were mixed with tumor cells before injection at the same site, but no suppression was observed when bacteria and tumor cells were injected at separate sites (table 4).

Effect of ATS on Suppression of Murine Fibrosarcoma Transplants With Heat-Killed LM

Another requirement for BCG-mediated suppression of tumor growth is the ability of the host to respond immunologically to mycobacterial antigens (1). Mice treated with ATS, cortisone acetate, or thymectomy and sublethal irradiation were unable to suppress the growth of fibrosarcoma transplants at sites of infection with BCG (19). LM might suppress the growth of fibrosarcoma transplants by nonimmunologic mechanisms. Direct contact between tumor cells and LM is required for suppression of tumor growth. Hemolytic and lipolytic cytotoxins have been isolated from LM (28) and these could be tumoricidal, particularly when tumor cells and bacteria are in close association.

Results from two types of experiments suggest that direct cytotoxicity does not account for all antitumor activity of heat-killed LM. In the first experiment, $10^6$ line-1038 tumor cells were mixed with $10^6$ heat-killed LM and incubated in 1 ml HBSS-FCS for 2 hours at 24°C. At the beginning of incubation, 95.5% of the tumor cells excluded 0.13% trypan blue.

**Table 1.—Suppression of growth of murine fibrosarcoma transplants at sites of injection with viable LM or BCG**

<table>
<thead>
<tr>
<th>Number of line-1038 tumor cells</th>
<th>Number of viable organisms</th>
<th>Tumor incidence $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$5 \times 10^3$</td>
<td>$10^2$</td>
<td>0/12/30 12/29/30</td>
</tr>
<tr>
<td></td>
<td>$10^3$</td>
<td>2/27/40 24/30/30</td>
</tr>
<tr>
<td></td>
<td>$10^4$</td>
<td>3/34/40 27/30/30</td>
</tr>
<tr>
<td></td>
<td>$10^5$</td>
<td>17/38/40</td>
</tr>
<tr>
<td></td>
<td>$10^6$</td>
<td>20/40/40</td>
</tr>
<tr>
<td></td>
<td>$10^7$</td>
<td>29/30/30</td>
</tr>
<tr>
<td></td>
<td>$10^8$</td>
<td>28/30/30</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>35/36/40</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>25/30/30</td>
</tr>
</tbody>
</table>

$^a$ Either $5 \times 10^6$ or $5 \times 10^8$ line-1038 tumor cells mixed with viable LM or BCG immediately before id inoculation of nonimmune mice.

$^b$ Number of mice with tumors/No. surviving infection/No. inoculated. Data combined from 4 expts. with similar results. Bold values differ significantly from controls at $P<0.01$ by the chi-square test.

$^c$ $5 \times 10^6$ or $5 \times 10^8$ line-1038 tumor cells in HBSS-FCS used for injection.

$^d$ $5 \times 10^8$ line-1038 tumor cells in TSB used for injection.

### Figure 1

**Text-figure 1.—Suppression of line-1038 fibrosarcoma growth with viable LM (circles) or viable BCG (squares).** Bacteria mixed with $5 \times 10^9$ (closed symbols) or $5 \times 10^8$ (open symbols) line-1038 cells before id injection into nonimmune syngeneic recipients. Percent tumor incidence computed from number of tumors that developed in mice surviving 2 weeks after injection of bacteria-tumor cell mixtures.
Text-figure 2.—Suppression of line-1038 fibrosarcoma growth with viable (○—○) or heat-killed (▲—▲) LM. Percent tumor incidence computed as in text-figure 1.

Table 2.—Suppression of growth of murine fibrosarcoma transplants with heat-killed LM a

<table>
<thead>
<tr>
<th>Number of organisms</th>
<th>Tumor incidence b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt. 1</td>
</tr>
<tr>
<td>10⁶</td>
<td>1/10/10</td>
</tr>
<tr>
<td>10⁷</td>
<td>2/10/10</td>
</tr>
<tr>
<td>10⁸</td>
<td>5/10/10</td>
</tr>
<tr>
<td>10⁹</td>
<td>6/10/10</td>
</tr>
<tr>
<td>10¹⁰</td>
<td>9/10/10</td>
</tr>
<tr>
<td>10¹¹</td>
<td>7/10/10</td>
</tr>
<tr>
<td>10¹²</td>
<td>10/10/10</td>
</tr>
<tr>
<td>0</td>
<td>9/10/10</td>
</tr>
</tbody>
</table>

a 5×10⁶ line-1038 tumor cells mixed with heat-killed LM immediately before id injection.

b Number of mice with tumors/No. surviving infection/No. inoculated. All animals survived >2 wk after injection. Italics values differ from controls at P<0.05. Bold values differ at P<0.01.

c Controls received 5×10⁷ line-1038 tumor cells in HBSS-FCS.

Table 3.—LM-mediated suppression of murine fibrosarcoma transplants in mice immunized to LM and in nonimmune mice a

<table>
<thead>
<tr>
<th>Number of organisms</th>
<th>Tumor incidence b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immune recipients</td>
</tr>
<tr>
<td>10⁶</td>
<td>1/18/20</td>
</tr>
<tr>
<td>10⁷</td>
<td>3/18/20</td>
</tr>
<tr>
<td>10⁸</td>
<td>13/10/20*</td>
</tr>
<tr>
<td>10⁹</td>
<td>6/10/10</td>
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<tr>
<td>10¹⁰</td>
<td>7/10/10</td>
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<tr>
<td>10¹¹</td>
<td>10/10/10</td>
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<td>10¹²</td>
<td>10/10/10</td>
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<tr>
<td>0</td>
<td>9/9/10</td>
</tr>
<tr>
<td>10¹³</td>
<td>10/10/10*</td>
</tr>
<tr>
<td>10¹⁴</td>
<td>17/18/20*</td>
</tr>
</tbody>
</table>

a 5×10⁶ line-1038 tumor cells mixed with viable LM immediately before id injection into nonimmune mice or mice that had received 10⁶ LM organisms 11 days earlier.

b Number of mice with tumors/No. surviving infection/No. inoculated. Data combined from 2 experiments, with similar results. Italicized values differ from controls at P<0.05; bold values differ from controls at P<0.01.

c Tumor incidence in immune recipients differs from that in nonimmune recipients at P<0.009.

d 5×10⁶ line-1038 tumor cells in HBSS-FCS used for injection.

e 5×10⁶ line-1038 tumor cells in TSB used for injection.

After 2 hours, cells incubated with LM were 87.9% viable, whereas cells incubated with control medium were 88.6% viable. Lack of acute toxicity in vitro does not rule out the possibility of direct toxicity exerted over a longer period in vivo. A second experiment was designed to elucidate events in vivo. Mice were treated with ATS before injection of tumor cells with or without addition of heat-killed LM. Additional groups were treated with NRS or were not treated prior to injection. Treatment with ATS abolished the antitumor activity of LM (table 5). NRS increased the number of tumors developing from mixtures of LM and tumor cells relative to untreated controls (P<0.002). However, ATS was significantly more effective than the NRS (P<0.002).

Regression of Established Murine Fibrosarcoma Transplants

The observation that complete suppression of line-1038 tumor cells could be produced at the site of infection with viable LM suggested that established transplants might also be susceptible to the antitumor activity of LM. Mice were given an id injection of

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A significant reduction in tumor incidence was achieved at 3 days when nodules averaged 1 mm in diameter, but not at 7 or 11 days when tumors averaged 4.7 and 9.7 mm, respectively (table 6). In a subsequent experiment, regression of 5-day tumor nodules measuring 2.2 mm in diameter resulted from an intratumor injection of $10^7$ LM (table 7). Multiple intratumor injections of viable LM were no more effective than a single dose (table 7). In the same experiment, 10$^7$ BCG in 0.1 ml medium 7H9 did not produce a significant number of regressions when compared to a group receiving medium 7H9 alone.

In attempts to augment the antitumor activity (table 8) of LM a combination of BCG and LM was no more effective than LM alone in producing regression of 5-day tumor transplants; a tenfold increase in the number of LM injected failed to increase the number of regressions; and preimmunization with an iv dose of 10$^3$ LM prevented deaths from an intratumor injection of $10^3$ LM, but did not augment the antitumor activity of the organisms. Intratumor injection of 10$^7$ BCG in 0.1 ml medium 7H9 did not produce a significant number of regressions of 3-day tumor transplants. Intratumor injection of 10$^6$ viable LM into BCG-immune recipients a

Table 6.—Regression of established murine fibrosarcoma transplants. Effect of intratumor injection of viable LM at different times after tumor cell injection. *

<table>
<thead>
<tr>
<th>Day</th>
<th>Tumor diameter (mm)</th>
<th>Tumor incidence b</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.0</td>
<td>12/20/20</td>
</tr>
<tr>
<td>7</td>
<td>4.7</td>
<td>16/20/20</td>
</tr>
<tr>
<td>11</td>
<td>9.7</td>
<td>7/20/20</td>
</tr>
</tbody>
</table>

* 5 X 10$^6$ line-1038 tumor cells injected id into nonimmune recipients. After 3, 7, and 11 days, 0.1 ml TSB or 10$^6$ viable LM in equal volume of medium was injected directly into tumor site.

b Number of mice with tumor/No. surviving infection/No. inoculated.

Lack of Systemic Tumor-Specific Immunity in Mice Receiving Tumor Cells and LM

Animals surviving suppression and regression experiments were rechallenged with 5 X 10$^6$ line-1038 tumor cells. Mice receiving intratumor injections of LM or iv injections of LM and tumor cells were no more resistant to tumor challenge than were mice that had not received previous injections of LM or tumor cells. Tumor incidence was greater than 90% in all groups.

**DISCUSSION**

Local infection with LM prevented growth of a murine fibrosarcoma and produced regression of established fibrosarcoma transplants. In suppression and regression experiments, the antitumor activity
of LM was superior to that of BCG. The virulence of LM, however, limited its utility as an immunotherapeutic agent. While $10^7$ BCG organisms were well tolerated, the intratumor or id injection of $10^5$–$10^6$ LM killed 15–60% of recipients. Attempts were made to eliminate the lethality of a virulent infection by use of heat-killed LM and by immunization of recipients with a sublethal dose of viable organisms. Mixing of heat-killed LM with tumor cells before injection into mice suppressed tumor growth, but intratumor injection of heat-killed LM produced no tumor regression. Immunization of recipients with an iv dose of LM prevented the mortality associated with the injection of $10^7$–$10^8$ viable LM without affecting the immunotherapeutic activity of LM infection.

Preimmunization of the host to LM was not required for LM-mediated tumor suppression or regression. Youdim et al. (7) reported that growth of a murine fibrosarcoma could be prevented if mixtures of LM and tumor cells were injected into the foot pads of mice already immune to LM. Preliminary observations by our group (29) and Youdim (30) indicated that LM-mediated suppression of tumor growth could occur in nonimmune recipients. In this report we have compared tumor growth in LM-immune and nonimmune mice that received mixtures of LM and tumor cells. The dose of LM was varied from 1 to $10^7$ organisms, and no consistent difference in tumor incidence was noted between LM-immune and nonimmune recipients.

The antitumor activity of LM cannot be attributed to the trauma of intratumor injection or to the toxicity of the medium in which the LM was grown. The incidence of tumors developing from mixtures of tumor cells and TSB was indistinguishable from that of tumors growing from suspensions of tumor cells in HBSS-FCS. Intratumor injection of TSB or medium 7H9 reduced the incidence of tumors in some experiments, but the effect was never statistically significant. LM could have exerted a direct toxic effect on tumor cells (28), but against this possibility are two lines of evidence: Mixture of heat-killed LM with tumor cells in vitro did not reduce tumor cell viability; a similar lack of direct toxicity was observed when living LM organisms were mixed with murine fibrosarcoma cells (7). Suppression of the host’s immune response with ATS permitted the growth of tumors from mixtures of heat-killed LM and tumor cells. Treatment of mice with ATS inhibited the development of a primary immune response to viable LM (31). Transfer of resistance to LM with spleen cells from LM-immune mice was abrogated by ATS treatment of the cells or the recipient (32, 33). Elimination of the antitumor activity of heat-killed LM by ATS suggests, but does not establish, that killing of tumor cells resulted from an immune reaction to the LM.

Several parallels exist between the antitumor activity of LM and that of BCG. Treatment with ATS permitted the growth of tumors from mixtures of BCG and fibrosarcoma cells (19). Growth of murine fibrosarcomas was suppressed with viable BCG in BCG-immune and nonimmune recipients (17). Heat-killed BCG also suppressed murine tumor growth, but when equal numbers of organisms were compared, viable BCG was more effective. Local infection with BCG did not augment systemic tumor-specific immunity to murine fibrosarcomas (17). Close contact between BCG and tumor cells was required for suppression of line-1038 fibrosarcoma growth (17). Preimmunization with BCG or injection of BCG at a separate site did not affect growth of this tumor. However, the ability of mouse fibrosarcoma transplants to grow in BCG-infected hosts varied among different tumor lines (34). Similarly, inhibition of tumor growth after systemic infection with LM might be observed with tumor lines other than 1038.

LM and BCG are obligate intracellular parasites rapidly, phagocytized by reticuloendothelial cells of the infected host. Elimination of both organisms requires the presence of activated macrophages. Macrophage activation, in turn, depends on the development of a population of specifically immune, short-lived, thymus-derived lymphocytes (21). Once activated, macrophages of BCG-immune animals can destroy various intracellular pathogens. Macrophages from mice infected with BCG (35, 36) or LM (37) can either kill or inhibit the growth of tumor cells in vitro. Macrophages and histiocytes have been observed in close association with degenerating tumor cells in vivo after intratumor injection of BCG in guinea pigs (38). These observations suggest that tumor cells may be killed by macrophages as “innocent bystanders” at sites of infection with LM or BCG. While this is an appealing model, it discounts the possible role of lymphocytes as direct effectors of tumor-cell killing. Specifically sensitized thymus-derived lymphocytes accumulate at sites of inflammation induced by LM or BCG. Although these cells are not bactericidal, lymphocytes from BCG-immune animals stimulated with purified protein derivative of tuberculin can kill tumor cells either by direct contact (35) or the release of tumoricidal lymphokines (39, 40). It is not known whether lymphocytes from LM-infected mice are similarly tumoricidal. To date it has been difficult to design experiments to delineate the relative contribution of lymphocytes and macrophages to killing of tumor cells at sites of bacterial infection. Observation of the antitumor activity of LM may facilitate these studies.

LM was more effective than BCG in producing suppression and regression of line-1038 tumor transplants. This apparent difference in antitumor activity may relate to the rapid proliferation of LM and to the early development of anti-LM immunity. After iv injection, the number of LM organisms in the liver and spleen of mice increases by 2–3 logs within 3 days (41). From 12 to 14 days are required for a 1- to 2-log increase in the number of BCG organisms after iv administration (41). The cellular immune response to LM is maximal 6 days after iv injection (42), whereas an optimal response to BCG requires 2 weeks to develop (43). Comparable differences in the kinetics of bacterial proliferation and host response would be anticipated after id injection of LM and
The antitumor activity of LM and BCG appeared to depend on the number of organisms administered (tables 1, 2; text-figs. 1, 2). For comparison of antitumor activity, equal numbers of CFU were injected. Within a few days, substantial differences could have developed in the number of LM and BCG organisms at the site of infection. Complete suppression of tumor growth was observed when tumor cells were mixed with 10^7 viable LM or 10^9 heat-killed LM before injection, but only partial suppression occurred with 10^7 viable BCG. Technical difficulties in concentrating BCG suspensions prevented the injection of a larger number of organisms, but complete suppression of tumor growth might have been produced had this been possible. Regression of fibrosarcoma transplants 5 days after the injection of tumor cells was accomplished by the intratumor injection of LM. Intratumor injection of BCG was no more effective than intratumor injection of diluent. Again, proliferation of LM may have been important. BCG-mediated regression of established line-1038 transplants has, however, been observed in mice previously immunized to BCG (17). The anamnetic response to mycobacteria develops more rapidly than a primary response. Rapid development of an immune response to LM or to BCG may be important for the elimination of a constantly increasing tumor burden.

Studies of the effects of LM infection on other murine tumor systems may be informative. Sublethal doses of LM administered iv are eradicated by host macrophages in the liver and spleen. Data presented in this paper and in other reports (7, 16, 17, 27) suggest that tumor cells are killed when in contact with leukocytes responding to bacterial infection. Murine tumors that localize in the liver and spleen might be treated effectively by iv LM infection. Candidates for such treatment include hepatomas, leukemias, and lymphomas.

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