Mechanisms of *Listeria monocytogenes*-Mediated Modulation of Tumor Growth

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Studies using *Listeria monocytogenes* as an antitumor agent were initiated to determine the requirements for *Listeria*-mediated tumor inhibition to occur. When Strain 13 guinea pigs were injected with an admixture of viable *Listeria* and a methylcholanthrene-induced fibrosarcoma in a ratio of 1 bacterium to 100 tumor cells, *Listeria* had a marked capacity to inhibit tumor growth. This confirms an earlier study in our laboratory (M. M. Dustoor, A. Fulton, W. Croft, and A. A. Blazkovec, Infect. Immun. 23:54–60, 1979). At no time did animals exhibit overt symptoms of disease as a result of *Listeria* infection. Animals treated with antilymphocyte serum, which had previously been shown to abrogate T-cell functions, were no longer able to suppress *Listeria*-tumor cell mixtures. Treatment in vivo with carrageenan, a macrophage-inhibitory agent, also abrogated *Listeria*-mediated tumor inhibition. These results suggest that *Listeria*-mediated inhibition requires intact T-lymphocyte and macrophage function. Experiments in which *Listeria* was given in admixture with the tumor cells or in the opposite flank demonstrated that the antitumor effects require intimate association of the *Listeria* and tumor cells. Histopathological studies, showing that macrophages and lymphocytes are the predominant inflammatory cells present at sites of tumor destruction, further suggest a role for these cells in *Listeria*-mediated inhibition. Animals which had rejected prior *Listeria*-tumor cell inocula were resistant to rechallenge with the homologous tumor for more than 1 year. This work thus confirms in vitro studies demonstrating that both lymphocytes and macrophages are required for *Listeria*-mediated tumor inhibition to occur. This study demonstrates that viable *Listeria* can have potent antitumor effects without causing overt disease as a result of *Listeria* infection.

In recent years, a great deal of interest has developed in the use of bacterial agents as therapeutic tools in the treatment of malignant disease. Much effort has been directed toward the study of Bacillus Calmette-Guérin (BCG) and *Corynebacterium parvum* as immunotherapeutic agents. Both organisms have produced some promising results in clinical trials, most notably in the treatment of malignant melanoma. Neither organism is effective in the treatment of all tumor types, and in some cases adverse side effects occur.

*Listeria monocytogenes*, another intracellular parasite, has only recently been examined for possible antitumor properties. Youdim and co-workers (14–18) have reported that *Listeria* treatment can retard and in some cases completely prevent the growth of several murine tumors. Bast et al. (1) also have reported that *Listeria* has murine tumor inhibitory properties when admixed with tumor inocula and can inhibit small established tumors when given intrasessionally, but significant numbers of animals were seen to succumb to fatal *Listeria* infection. Bast et al. (2) also demonstrated suppression of growth of line 10 hepatoma cells when admixed with viable *Listeria* before injection into Strain 2 guinea pigs but, again, mortality due to *Listeria* infection was a major problem. They were not able to induce significant regression of established tumors treated intrasessionally with *Listeria*.

Youdim and co-workers (14, 16, 18) carried out several studies to characterize the cell type(s) responsible for *Listeria*-mediated tumor killing in a murine system and found that tumor killing, in vitro, requires both lymphocytes sensitized to *Listeria* antigens and macrophages from normal or *Listeria*-immune animals.

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It has been reported previously in this laboratory (4) that *Listeria* has potent antitumor effects in Strain 13 guinea pigs bearing a syngeneic methylcholanthrene (MCA)-induced fibrosarcoma. These inhibitory effects were expressed when *Listeria* was admixed with tumor cells before transplantation or when it was injected into established tumor nodules. Animals previously rejecting tumor-*Listeria* mixtures displayed enhanced skin test reactivity to a tumor extract as compared to animals rejecting untreated tumors. No significant mortality as a result of *Listeria* infection was ever observed.

The purpose of this study was to investigate the possible mechanisms of *Listeria*-mediated tumor inhibition using Strain 13 guinea pigs bearing an MCA-induced fibrosarcoma.

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MATERIALS AND METHODS

**Experimental animals.** Inbred Strain 13 guinea pigs were obtained from a colony originally supplied by the National Institutes of Health (Bethesda, Md.) and now maintained at the breeding facilities of the University of Wisconsin-Madison. All animals weighed between 500 and 800 g at the time of use.

**Organisms.** The strain of *L. monocytogenes* used in these studies was originally obtained from D. W. Smith, Department of Medical Microbiology, University of Wisconsin-Madison. The growth characteristics and maintenance of this organism have been described previously (6).

**Tumor.** The two fibrosarcomas used in this study, designated MCA-1 and MCA-2, were originally induced in this laboratory by injecting Strain 13 guinea pigs subcutaneously with 20 mg of 3-methylcholanthrene in 0.25 ml of corn oil. The induction and maintenance of these tumors has been described previously (4). The MCA-1 tumor used in this study was in transplant generation 21. It had been maintained by several intraperitoneal transplantations in Strain 13 guinea pigs. The MCA-2 tumor was in transplant generation 4.

**Subcutaneous injection of tumor cells and viable *Listeria* and measurement of tumor growth.** Tumor cells prepared as described and maintained in liquid nitrogen were thawed, washed two times, and suspended to the desired concentration in medium 199 (M-199); 0.2 ml was injected subcutaneously in the central abdominal area using a 20 gauge, 1-inch (ca. 2.54-cm) needle.

In experiments where *Listeria* were injected with the tumor cells, a portion of stock suspension containing a known quantity of viable *Listeria* was thawed, washed, and diluted to the desired concentration in M-199. Since equal volumes of the tumor cell suspension and *Listeria* suspension were combined before injection, both suspensions were prepared to contain twice the desired number of tumor cells and *Listeria*.

The animals were monitored for tumor growth at regular intervals over a 6- to 12-month period. The size of the tumor was then expressed as the mean tumor diameter based on two perpendicular tumor diameter measurements.

**ALS.** Heterologous antisera were prepared as previously described by Schell et al. (11). Each of eight rabbits was injected intravenously with 2 ml of saline suspension containing 10⁶ thymocytes obtained from newborn Strain 13 guinea pigs. Injection was via the marginal ear vein. Two weeks later each rabbit was reinjected intravenously with 2 ml of a saline suspension containing 10⁶ thymocytes. One week after the last injection, rabbits were exsanguinated. Antisera were prepared and stored at −20°C until fractionated.

The antilymphocyte serum (ALS) and normal rabbit serum (NRS) were fractionated by precipitation with ammonium sulfate. After exhaustive dialysis against saline, the fractions were sterilized by filtration, and total protein content was determined as previously described (11).

**Titration of ALS.** After absorption of ALS with guinea pig erythrocytes, leukoagglutinin levels were determined as previously described (11). Serial twofold dilutions of the fractions were prepared using M-199 containing 10% NRS. A 0.1-ml sample of cell suspension containing 2 × 10⁶ guinea pig thymocytes suspended in saline was added to each dilution. After incubating overnight at 4°C, contents of each tube were examined microscopically for the presence of agglutinated cells. The highest dilution of each fraction showing leukoagglutination was chosen as the endpoint titer. Volume adjustments were then made so that each fraction pool contained approximately 12 to 15 mg of protein per ml and a leukoagglutinin titer of approximately 1:10,000.

**Preliminary assessment of the immunosuppressive capacity of each fraction was carried out as follows.** Guinea pigs previously vaccinated with BCG were skin tested with 2.5 μg of purified protein derivative. Those animals showing strong delayed-type hypersensitivity reactions were chosen for treatment with ALS. Animals were given a 2.0-ml intraperitoneal injection of fractionated antiserum each day for 7 consecutive days. On day 7, the animals were skin tested again with 2.5 μg of purified protein derivative. Only those ALS fractions capable of significant suppression of delayed-type hypersensitivity were used in the experiments described in this study.

**ALS treatment.** Animals were injected intraperitoneally with 2.0-ml samples of fractionated ALS, NRS, or saline each day for 6 consecutive days. On day 7, animals were injected with various tumor-*Listeria* mixtures. Antiserum treatment was continued every other day for 10 subsequent days (five injections).

**Carrageenan treatment.** In an attempt to impair the reticuloendothelial system function, animals were treated with carrageenan. Carrageenan, type 1, derived from Irish moss (Sigma Chemical Co., St. Louis, Mo.), was dissolved in sterile physiological saline at a concentration of 10 mg/ml and sterilized by autoclaving for 10 min at 15 lb/in². Two days before tumor transplantation and on the day of transplantation, guinea
Pigs were injected intraperitoneally with 50 and 150 mg of carrageenan, respectively. All carrageenan-treated and normal control animals were then injected with $2.0 \times 10^6$ MCA-1 cells with or without $2.5 \times 10^4$ viable Listeria.

**Histology.** Tissues were fixed in buffered Formalin and sectioned at 5 μm. They were stained with hematoxylin and eosin and observed by light microscopy.

**Statistical analysis.** To evaluate the data concerning tumor size and mean survival time, comparisons between the control and Listeria-treated groups were carried out using Student’s t test as described by Snedecor et al. (13). To test the statistical significance of proportions in two independent samples, the chi-square ($\chi^2$) test was used.

**RESULTS**

**Growth pattern of MCA-1 tumor and effect of L. monocytogenes on tumor growth.** When Strain 13 guinea pigs were injected subcutaneously with $2.5 \times 10^6$ MCA-1 tumor cells, the resulting tumors grew progressively in five of seven animals (Table 1). In animals injected with a mixture of $2.5 \times 10^6$ MCA-1 cells and $2.5 \times 10^4$ viable Listeria, the tumors grew progressively for the first 7 days and then regressed completely. All seven animals were tumor free 14 days after injection of the Listeria-tumor cell mixture, demonstrating significant tumor inhibition ($P < 0.01$). The mean tumor diameter in Listeria-treated animals was slightly larger than in the group receiving tumor alone at days 3 and 7 posttransplantation, and this is probably due to the Listeria-induced influx of inflammatory cells. All animals in which tumor regression occurred remained free of tumor for 1 year, after which time they were used for the specificity experiment discussed below. The mean survival time of animals bearing progressively growing tumors without Listeria treatment was 58.6 days. At no time did animals receiving Listeria-tumor mixtures exhibit overt symptoms of disease due to Listeria infection. Upon gross examination, the injection site of tumor-Listeria-mixture was erythematous and indurated for 3 to 4 days after injection. This confirms a previous study in this laboratory (4) showing marked tumor inhibition at Listeria-tumor cell rates of 1:100.

**Presence of specific antitumor immunity in guinea pigs receiving Listeria-tumor cell mixtures.** To determine whether specific tumor immunity had developed, normal guinea pigs and guinea pigs previously rejecting Listeria-tumor cell mixtures 12 to 14 months earlier were challenged with $2.0 \times 10^6$ MCA-1 or MCA-2 cells. Table 2 shows that progressive tumor growth occurred in 100% of the normal animals given MCA-1 cells (group 1). All animals succumbed to the tumor with a mean survival time of 58.2 days. In guinea pigs previously immunized to the MCA-1 tumor (group 2), the MCA-1 challenge inoculum initially grew in all eight animals but had completely regressed in all animals by 24 days postinjection.

To determine whether the tumor immunity was specific for an MCA-1 tumor challenge, normal or MCA-1-immune guinea pigs were challenged with the MCA-2 tumor. In normal animals injected with $2.0 \times 10^6$ MCA-2 cells (group 3), the tumors grew progressively in four of the seven animals, leading to death of the recipients at a mean time of 64.7 days. Ten of ten guinea pigs immune to the MCA-1 tumor were able to completely inhibit the growth of the MCA-2 challenge (group 4). Likewise, the three animals previously immunized to the MCA-2 tumor were immune to challenge with the MCA-1 tumor (group 5). These results suggested either (i) that immunization with the MCA-1 or MCA-2 tumor cells induces nonspecific tumor immunity, or (ii) that the MCA-1 and MCA-2 tumors share a cross-reacting antigen.

**Effect of ALS treatment in Listeria-mediated tumor inhibition.** To determine

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<th>Table 1. Tumor incidence in guinea pigs injected with MCA-1 tumor cells in admixture with Listeria</th>
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<td><strong>Group</strong></td>
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<td>Tumor cells (2.5 × 10^6)</td>
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* Mean tumor diameter ± standard error for groups of seven Strain 13 guinea pigs each injected with 2.5 × 10^6 MCA-1 tumor cells alone or admixed with 2.5 × 10^4 viable *L. monocytogenes*. Parentheses indicate number of guinea pigs with tumors per number of inoculated survivors.

* Mean survival time for animals bearing tumors.
whether lymphocyte function is required for *Listeria*-mediated inhibition to occur, Strain 13 guinea pigs were treated with ALS, NRS, or saline. All animals received daily intraperitoneal injections of 2.0 ml of ALS, NRS, or sterile saline on 6 consecutive days, after which all animals received either 2.0 x 10^6 MCA-1 cells alone or 2.0 x 10^6 MCA-1 cells admixed with 2.5 x 10^4 viable *Listeria*. The serum treatments were continued every other day for 10 more days.

Most of the animals receiving ALS, NRS, or saline and subsequently injected with MCA-1 alone developed progressively growing tumors (Table 3). In animals receiving saline prior to administration of MCA-1 and *Listeria*, the tumor was completely inhibited. In animals receiving NRS treatment, one animal did develop a progressively growing tumor even with *Listeria* administration. In contrast, ALS-treated animals were not able to inhibit the growth of the tumor-*Listeria* inoculum. The only group in which complete suppression of the tumor occurred was the saline-treated group receiving MCA-1-*Listeria* mixtures.

**Table 2. Tumor immunity in guinea pigs immune to MCA-1 or MCA-2 tumor cells**

| Group | MCA-1 immune | MCA-2 immune | Challenge tumor (2.0 x 10^6 cells) | Mean tumor diameter (mm) on day:
|-------|-------------|-------------|-----------------------------------|-------------------
|       |             |             | 3      | 7      | 14     | 24     | 32     | 46     |
| 1     | No          | No          | MCA-1  | 2.4 ± 0.2 | 3.8 ± 0.5 | 11.2 ± 0.2 | 25.4 ± 3.1 | 32.4 ± 3.0 | 45.1 ± 7.2 |
| 2     | Yes         | No          | MCA-1  | 5.4 ± 1.3 | 7.0 ± 0.3 | 0.3 ± 0.3 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|       |             |             | (8/8)  | (8/8)  | (1/8)  | (0/8)  | (0/8)  | (0/8)  | (0/8)  |
| 3     | No          | No          | MCA-2  | 2.2 ± 0.9 | 7.5 ± 1.2 | 12.4 ± 1.6 | 15.9 ± 4.7 | 19.2 ± 6.7 | 28.2 ± 10.4 |
|       |             |             | (4/7)  | (7/7)  | (7/7)  | (6/7)  | (5/7)  | (4/7)  | (4/7)  |
| 4     | Yes         | No          | MCA-2  | 2.3 ± 0.5 | 6.0 ± 0.9 | 0.8 ± 0.3 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|       |             |             | (7/10) | (10/10) | (4/10) | (0/10) | (0/10) | (0/10) | (0/10) |
| 5     | No          | Yes         | MCA-1  | 5.5 ± 0.5 | 8.3 ± 1.2 | 1.6 ± 1.6 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|       |             |             | (3/3)  | (3/3)  | (1/3)  | (0/3)  | (0/3)  | (0/3)  | (0/3)  |

*Mean tumor diameter ± standard error for groups of Strain 13 guinea pigs. Animals were normal controls, MCA-1 immune, or MCA-2 immune, and were challenged with 2.0 x 10^6 MCA-1 or 2.0 x 10^6 MCA-2 tumor cells. Parentheses indicate number of guinea pigs with tumors per number of inoculated survivors.*

**Table 3. Effect of ALS in guinea pigs injected with MCA-1 tumor cells in admixture with *Listeria***

| Group | Serum | Viable Listeria (2.5 x 10^4) | Mean tumor diameter (mm) on day:
|-------|-------|-------------------------------|-------------------
|       |       | 3    | 7    | 12   | 21   | 28   | 35   | 42   |
| 1     | Saline| Yes  | 10.7 ± 0.4 | 8.9 ± 1.0 | 1.9 ± 1.4 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 2     | Saline| No   | 6.0 ± 0.5 | 8.5 ± 0.5 | 13.1 ± 1.1 | 19.2 ± 2.3 | 24.5 ± 4.0 | 28.5 ± 4.0 | 32.8 ± 6.7 |
| 3     | NRS   | Yes  | 7.0 ± 1.2 | 7.3 ± 0.5 | 5.2 ± 0.9 | 1.3 ± 2.9 | 2.4 ± 5.4 | 3.6 ± 8.0 | 3.9 ± 8.8 |
|       |       | (6/6) | (6/6)  | (6/6)  | (1/6)  | (1/6)  | (1/6)  | (1/6)  | (1/6)  |
| 4     | NRS   | No   | 4.1 ± 0.7 | 6.9 ± 0.6 | 11.1 ± 0.6 | 17.0 ± 3.7 | 18.7 ± 5.5 | 20.7 ± 7.8 | 24.4 ± 8.9 |
| 5     | ALS   | Yes  | 7.7 ± 0.4 | 6.3 ± 0.4 | 3.1 ± 0.4 | 8.6 ± 2.3 | 23.1 ± 2.3 | 28.8 ± 1.4 | 34.6 ± 3.2 |
| 6     | ALS   | No   | 5.4 ± 0.6 | 5.6 ± 0.6 | 11.5 ± 1.2 | 27.2 ± 1.8 | 38.7 ± 3.1 | 41.6 ± 1.6 | 42.8 ± 5.4 |

*Mean tumor diameter ± standard error for groups of Strain 13 guinea pigs treated with ALS, NRS, or saline and then injected with 2.0 x 10^6 MCA-1 cells either alone or admixed with 2.5 x 10^4 viable *L. monocytogenes*. Parentheses indicate number of guinea pigs with tumors per number of inoculated survivors.*
growth rate than animals not receiving Listeria, all of these animals did develop progressively growing tumors.

Requirement for intimate association of Listeria and tumor cells. To determine whether the efficacy of Listeria is dependent on close proximity of bacteria with tumor cells, the following experiment using three groups of six to eight animals each was carried out. The first group received $2.0 \times 10^6$ MCA-1 cells admixed with $2.5 \times 10^4$ Listeria and injected on the right flank. The second group received $2.0 \times 10^6$ MCA-1 cells injected on the right flank and $2.5 \times 10^4$ Listeria on the left flank. The third group received $2.0 \times 10^6$ MCA-1 cells on the right flank and 0.2 ml of sterile saline on the left flank. Figure 1 shows that tumors grew progressively in all animals receiving tumor and saline injections or tumor and Listeria given on opposite flanks. Tumor inhibition was seen only in the group injected with tumor cells admixed with Listeria. In this group, four of the six animals showed complete inhibition, although one tumor did not completely regress until 66 days after transplantation. These results differed significantly from the group receiving Listeria opposite the tumor inoculum ($P < 0.01$).

Histopathology of Listeria-injected tumor sites. To characterize Listeria-mediated tumor inhibition histopathologically, Strain 13 guinea pigs were injected with $2.5 \times 10^6$ MCA-1 cells. After the tumors were allowed to grow for 6 days, groups of animals were injected intraleesionally with either $5.5 \times 10^5$ viable Listeria or 0.2 ml of sterile saline. A third group received no further treatment. Animals were sacrificed at various times after the injections, and complete necropsies were carried out. Tissues were fixed, stained, and sections examined for evidence of infiltration by Listeria cells.
stained with hematoxylin and eosin, and observed by light microscopy.

Figure 2 shows the intense inflammatory reaction occurring 24 h after the intralesional injection of *Listeria* into an established tumor nodule. The upper part of the photograph is the dense tumor mass; the lower portion reveals the intense inflammatory reaction, consisting primarily of polymorphonuclear cells and a few lymphocytes. Figure 3 shows a higher magnification of the area shown in Fig. 2. Figure 4 shows the inflammatory reaction present 72 h after the injection of viable *Listeria* into an established tumor. Polymorphonuclear cells were still the predominant cell type in the inflammatory reaction site; in addition, many macrophages containing intracellular particles (arrow) and a few lymphocytes were seen. Untreated tumors revealed little or no inflammatory reaction. Saline-treated tumors revealed only a mild inflammatory reaction, consisting primarily of polymorphonuclear cells. Histological examination of animals previously rejecting *Listeria*-tumor inocula revealed no residual tumor cells at the injection site.

**DISCUSSION**

The results of this study have shown that *Listeria* has a marked ability to inhibit an MCA-induced fibrosarcoma in Strain 13 guinea pigs. When normal guinea pigs were injected with an admixture of viable *Listeria* and MCA-1 cells in a ratio of one bacterium to 100 tumor cells, all animals showed complete rejection of the tumor. Animals remained tumor-free for at least 1 year. In animals receiving tumor cells alone, 71% of the animals developed progressively growing tumors leading to death by day 78. At no time did animals receiving *Listeria*-tumor mixtures exhibit gross symptoms of disease as a result of *Listeria* infection. This confirms a previous study in our laboratory by Dustoor et al. (4) in which complete inhibition of tumor cell inocula by *Listeria* was observed. Bast et al. (2) showed complete inhibition of the line 10 hepatoma when admixed with viable *Listeria* and injected into Strain 2 guinea pigs. In this system, however, a high number of *Listeria* ($10^8$) was necessary to achieve inhibition of growth; this dose proved fatal to a large number of animals and
Fig. 3. Photomicrograph of a higher magnification of the area shown in Fig. 2. Many inflammatory cells, many of which are polymorphonuclear cells. A few lymphocytes are also seen. Hematoxylin and eosin. ×425.

Fig. 4. Photomicrograph of the inflammatory reaction occurring 72 h after the intralesional injection of Listeria. Many macrophages containing intracellular particles are visible (arrow) as well as a few lymphocytes and polymorphonuclear cells. Hematoxylin and eosin. ×425.
thus was a major limitation to the system. Bast et al. (1) also showed inhibition of murine tumors with *Listeria* treatment, but, again, as many as 60% of the mice succumbed to *Listeria* infection. The problems of toxicity due to *Listeria* infection have been avoided in this study by employing much lower numbers of viable *Listeria* (2.5 \times 10^6). We have never detected *Listeria* organisms systemically after subcutaneous injection.

Youdim et al. (17) demonstrated suppression of a murine MCA-induced tumor when tumor cells were admixed with viable *Listeria*. Prior immunity to *Listeria* was necessary for the tumor-inhibiting effects to be observed. In a later study, Youdim (14) found that injection of B16 melanoma cells admixed with *Listeria* resulted in progressive tumor growth in 50% of the animals compared to 87% of animals injected with tumor cells alone. In this system, prior immunity to *Listeria* was not necessary. Using a third system, Youdim (16) found that *Listeria* treatment of MCA-induced sarcomas in A/He mice led to growth of smaller tumors than those observed in mice given tumor alone, but no significant number of complete regressions occurred.

In the present study, animals previously rejecting MCA-1 cells or *Listeria*-MCA-1 mixtures were immune to rechallenge with the homologous MCA-1 tumor. All MCA-1-immune animals were also immune to challenge with the MCA-2 tumor, another MCA-induced fibrosarcoma induced in this laboratory in Strain 13 guinea pigs. Animals immune to the MCA-2 tumor likewise resisted a challenge with the MCA-1 tumor. This partially confirms the in vitro studies of Miller and Blazkovec (9) that lymph node lymphocytes from guinea pigs immune to the MCA-1 tumor were cytotoxic to MCA-2 target cells. In this in vitro system, however, MCA-2 immune lymphocytes were not cytotoxic to MCA-1 target cells. Thus, the two MCA-induced tumors appear to possess tumor-specific antigens as well as a common cross-reactive antigen, even though most chemically induced tumors have been shown usually to possess unique tumor-specific transplantation antigens (7). Prehn and Main (10) have also shown, however, that some MCA-induced sarcomas do cross-react.

The present study showed that carrageenan treatment abrogated the tumor-inhibiting effects of *Listeria*; this is probably attributable to carrageenan's macrophage-toxic properties, although its effects on coagulation, complement, etc., as causes of suppression cannot be ruled out at this time. That macrophages do play a role is supported by histological studies showing many macrophages at sites of *Listeria*-mediated tumor destruction.

Results of the present study confirm the in vitro studies of Youdim and Sharman (18), showing that macrophages and T lymphocytes are both required for tumor target cell killing in the presence of *Listeria*, and the in vivo study by Bast et al. (1), showing that the effects of administration of heat-killed *Listeria* are sensitive to ALS treatment.

Experiments in which *Listeria* and tumor cells were injected in admixture into the flank of each guinea pig or injected separately on opposite flanks show that the *Listeria* and tumor cells must be in close proximity to each other for tumor inhibition to occur, confirming the report of Youdim (14).

*Listeria*-mediated tumor inhibition thus seems to be similar to BCG-mediated regression in many ways. Both require viable organisms for optimal inhibition, close contact between the organism and the tumor cells is necessary, and the organisms are effective both when administered in admixture with the tumor inoculum and when injected into established tumor nodules of limited size; neither organism requires prior immunity, and both require an immunologically intact host for optimal results.

As with BCG inhibition, the mechanisms involved are not clearly understood. The most likely possibilities are that *Listeria* is directly toxic to tumor cells, or that tumor cells are destroyed as innocent bystanders by macrophages activated either directly by *Listeria* or via sensitized lymphocytes responding to *Listeria* antigens. *Listeria* could also have adjuvant properties permitting it to increase tumor-specific responses, thus leading to tumor destruction either directly by lymphocytes or by activated macrophages. The first possibility—namely, that *Listeria* is directly toxic to tumor cells—seems unlikely becauseadmixing *Listeria* and tumor cells in vitro does not cause a drop in viability, as determined by trypan blue exclusion.

The possibility exists that *Listeria* could mediate its effects by directly activating macrophages. *Listeria* and listerial cell wall antigens have been shown to act as murine B-cell mitogens (12) and to have adjuvant properties in increasing the antibody responses to both soluble and particulate antigens (3). The in vitro studies of Youdim and co-workers (15, 16, 18) and the current study indicate that macrophages are not directly activated by *Listeria* because lymphocytes are required for killing to occur both in vitro and in vivo. Neither study, however, determines which cell is the actual killer cell. Krahenbuhl and Remington (8) have demonstrated that purified macrophages from *Lis-
teria-infected mice can be induced to kill tumor target cells in vitro in the presence of Listeria antigens. In contrast, Youdim found that both adherent and nonadherent cells were required in vitro cultures for killing to occur. No role for antibody-mediated tumor inhibition has been identified. It is known from previous studies in our laboratory that little or no antibody to Listeria antigens appears after primary infection of guinea pigs (5). Antibodies to tumor antigens, however, might play a role in tumor growth inhibition and should be an area of future study. Due to the relative specificity of our ALS for T cell functions, however, we believe that it is most likely that T lymphocytes are required for in vivo tumor growth inhibition and that in vitro tumor cell killing is sensitive to anti-\( \theta \) serum treatment, as demonstrated by Youdim (16).

It is also plausible to speculate that the mechanism whereby enhanced tumor rejection occurs as late as 12 to 14 months after primary implantation of tumor cells admixed with Listeria involved T-cell function. Because of the persistence of apparent tumor immunity observed upon challenge with tumor cells in the absence of Listeria, it is possible that primary implantation with Listeria results in the persistence of Listeria-enhanced specific tumor immunity expressed in the form of specific T-cell function.

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LITERATURE CITED
