Immunotherapy of the Guinea Pig Line 10 Hepatocarcinoma with a Variety of Nonviable Bacteria

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ABSTRACT

A variety of heat-killed bacteria were tested for their capacity to induce regressions of established line 10 hepatocarcinomas in syngeneic guinea pigs. Multiple intraskeletal injections of heat-killed Escherichia coli, Streptococcus mutans, Listeria monocytogenes, and Propionibacterium acnes resulted in complete regression of the tumor in a majority of guinea pigs. Repeated injections of heat-killed Mycobacterium bovis strain Bacillus Calmette-Guérin caused no regressions. Surviving animals were immune to subsequent challenge with line 10 cells but not L2C cells, another syngeneic tumor.

INTRODUCTION

The line hepatocarcinoma of strain 2 guinea pigs has been extensively investigated as a model system to evaluate bacterial immunotherapy of tumors (8, 14). Viable BCG or subcellular components of BCG attached to mineral oil droplets have been shown to be therapeutically effective in eradicating established tumors that have metastasized (8, 14). Heat-killed BCG in the absence of oil droplets has little or no effect (1, 8, 14). In a recent study at this laboratory (5), multiple intraskeletal injections of heat-killed Staphylococcus aureus without oil into line 10 tumors similarly caused regressions of established tumors and induced tumor-specific immunity. Delerious side effects of viable bacteria or oil-containing bacterial preparations were thus avoided. We presently report that a variety of Gram-positive and Gram-negative heat-killed bacteria are also therapeutically effective against this tumor using a multiple injection protocol. The lack of effectiveness of heat-killed BCG was confirmed.

MATERIALS AND METHODS

Animals. Male and female strain 2 guinea pigs weighing 350 g or more were obtained from breeding colonies at the Frederick Cancer Research Center, Frederick, Md., and at this institution.

Tumors. The diethylnitrosamine-induced line 10 hepatocarcinoma of strain 2 guinea pigs was maintained in ascites form (7, 14). Line 10 cells in these experiments were of the sixth to 15th transplant generations. Injections of 10^6 viable line 10 cells i.d. into strain 2 guinea pigs invariably cause tumors which result in death of the recipients within 60 to 90 days (14). Tumors have been shown to metastasize to regional lymph nodes within 6 days after injection (12, 14).

Strain 2 L2C leukemia cells (Ia positive) were provided by Dr. A. Cooper, Tufts University School of Medicine, Boston, Mass. Injections of 3 x 10^6 L2C cells i.d. into normal animals resulted in fatal leukemia in 2 to 3 weeks; L2C-immune animals resist this challenge (5).

RESULTS

Guinea pigs were given injections i.d. in the right flanks with 10^6 line cells. When tumor diameters were approximately 1 cm (6 to 8 days), lesions were infiltrated with 0.3 to 0.4 ml of bacterial suspensions in 0.9% NaCl solution. Injections were repeated at weekly intervals for a total of 3 injections. Results are presented in Table 1. In summary, when sufficient numbers of bacteria were used, regressions were obtained in 12 of 15 animals treated with E. coli (1 to 5 x 10^11), 9 of 10 receiving injections of S. mutans (1 x 10^11), 24 of 31 given injections of L. monocytogenes (1 to 5 x 10^10), and 13 of 25 inoculate with P. acnes (1 to 5 x 10^9). Injections of lower numbers of bacteria were generally ineffective. Furthermore, no regressions were observed with multiple injections of BCG; these results are consistent with prior studies of others (1, 8, 14) demonstrating that heat-killed BCG induced little antitumoral effect in this system.

Lesion diameters usually increased to about 20 x 20 mm after the first bacterial injection, and small ulcerative areas appeared on lesion surfaces. Some animals became temporarily listless and experienced a small weight loss following injections of large numbers of L. monocytogenes. No side effects were observed in animals treated with other bacterial strains.
In animals that recovered, lesions began to diminish in size after the third bacterial injection and disappeared by about 50 days after onset of the experiments.

Recovered animals were challenged with $10^6$ line 10 cells i.d. into their contralateral flanks about 60 days after the initial injection of tumor cells. These animals were found to be resistant to this challenge and exhibited delayed cutaneous hypersensitive reactions to the line 10 cells. Subsequent challenges of line 10-resistant animals with $L_2C$cells were uniformly fatal. These results indicated that following bacterial-induced tumor regression, line 10-specific immunity was generated in these animals.

During the course of these studies, a number of other treatments were found to be ineffective: (a) single injections of bacteria into 6-day-old tumors resulted in no regressions; (b) no beneficial antitumor response was observed after multiple injections of bacteria into 14-day-old tumors; and (c) close contact between line 10 tumors and bacteria was essential since systemic inoculations of bacteria i. v., i.p., or i.d. into the contralateral flanks were not effective (data not shown).

**Table 1**

<table>
<thead>
<tr>
<th>Bacteria used for treatment</th>
<th>Experiment</th>
<th>No. of bacteria injection</th>
<th>No. of animals tumor free/no. challenged with</th>
<th>Line 10 cells</th>
<th>$L_2C$ cells</th>
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<tbody>
<tr>
<td><em>E. coli</em></td>
<td>1</td>
<td>$1 \times 10^4$</td>
<td>2/2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$1 \times 10^4$</td>
<td>5/5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>$1 \times 10^4$</td>
<td>3/6</td>
<td>2/2</td>
<td>0/2</td>
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<td><em>S. mutans</em></td>
<td>1</td>
<td>$1 \times 10^4$</td>
<td>1/7</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$1 \times 10^4$</td>
<td>5/5</td>
<td>5/5</td>
<td>0/2</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>1</td>
<td>$1 \times 10^4$</td>
<td>0/3</td>
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<td>ND</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$1 \times 10^4$</td>
<td>4/4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>$1 \times 10^4$</td>
<td>3/3</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td><em>P. acnes</em></td>
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<td>$1 \times 10^4$</td>
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<tr>
<td></td>
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<td>5/5</td>
<td>4/4</td>
<td>0/3</td>
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<tr>
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<td>3/3</td>
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<tr>
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<td>4/4</td>
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<tr>
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<td>$10^6$</td>
<td>0/4</td>
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<td>ND</td>
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<td>Untreated controls</td>
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<td></td>
<td>0/48</td>
<td>ND</td>
<td>ND</td>
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</table>

* Most animals surviving the initial procedures were rechallenged with $10^6$ line 10 cells alone after 2 months and 2 months later with $3 \times 10^5$ $L_2C$ cells.

DISCUSSION

In a prior report (5), we demonstrated that multiple injections of heat-killed *S. aureus* induced the regression of line 10 tumors. This observation has been extended in this study to a variety of other heat-killed Gram-negative and Gram-positive bacteria. In like manner, multiple injections of heat-killed *Candida albicans* have been shown to induce tumor regression (13).

The mechanism(s) by which multiple injections of heat-killed bacteria mediated the antitumor effects observed is not known. It was necessary for the bacteria to be injected at the site of tumor growth, indicating the importance of local effector mechanisms. However, systemic specific immunity was induced possibly as a consequence of processing of tumor antigens by macrophages and subsequent sensitization of lymphoid cells (2). *P. acnes* has been found to initiate a number of biological effector mechanisms including stimulation of cytotoxic macrophages (4) and natural killer cells (3). The other microorganisms could also stimulate these effector mechanisms. In this regard, human peripheral blood lymphocytes became cytoktoxic to a number of tumor cells following in vitro incubation with several soluble bacterial extracts including *L. monocytogenes* (10, 11). Induction of similar cells in vivo in the guinea pig could play a role in the observed tumor regression. Small amounts of BCG-derived peptidic substances combined with oil and adjuvants such as trehalose mycolate and muramyl dipeptid have recently been shown to cause regression of this tumor (9). An appropriate bacterial component in conjunction with other adjuvant properties of the bacteria tested may similarly have led to the antitumor effects observed in this study.

In summary, a variety of heat-killed bacteria were found to act as immunotherapeutic agents against the line 10 hepatocarcinoma of guinea pigs. This study indicates the need to examine the antitumor effects of multiple injections of sufficient numbers of these microbial agents against other tumors in other species.

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REFERENCES

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