Effect of *Myobacterium bovis* (Strain *Bacillus Calmette-Guérin*) on Macrophage Production by the Bone Marrow of Tumor-bearing Mice

Bernard Fisher, Stuart Taylor, Morton Levine, Elizabeth Saffer, and Edwin R. Fisher

Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213 [B. F., S. T., M. L., E. S.], and the Institute of Pathology, Shadyside Hospital Pittsburgh, Pennsylvania 15232 [E. R. F.]

### MATERIALS AND METHODS

All investigations utilized C3HeB/FeJ female mice, 8 to 12 weeks old. The tumor used was a spontaneous C3H mammary tumor maintained by serial implantation. A preliminary evaluation was made of the effect of BCG on macrophage colony formation by bone marrow cells from normal non-tumor-bearing mice sacrificed 2, 5, 7, 14, or 28 days following BCG or 0.9% NaCl solution inoculation. Subsequently, 3 additional experiments were carried out. Each used 4 groups of mice. Those in Group A were normal sham tumor-implanted, 0.9% NaCl solution-injected controls, and those in Group B were tumor-bearing, 0.9% NaCl solution-inoculated controls. Non-tumor-bearing sham-implanted mice in Group C and tumor-bearing mice in Group D were recipients of BCG 10 days after the tumor or sham-tumor was implanted. Tumor growth in mice of Groups B and D occurred following the s.c. implantation of 1-mm plugs of tumor into left hind legs distal to popliteal nodes. All mice in Experiment I were sacrificed 3 days after BCG or 0.9% NaCl solution inoculation and 13 days after tumor or sham-tumor implantation; in Experiment 2, those times were 11 and 21 days, and in Experiment 3, the times were 18 and 28 days, respectively. All animals were killed by cervical dislocation, were immediately immersed in antiseptic solution, and 1 femur was promptly removed by sterile technique from each of 3 mice in a group. Marrow cells were harvested as previously described (4). An aliquot of the pooled marrow cell suspension was counted and diluted so that there were 1 x 10^6 cells/ml. The suspension, 1.5 ml, was added to 9.6 ml of methylcellulose solution containing 2.25 ml of horse serum. One ml of the resulting suspension (containing 1.1 x 10^6 cells) was placed in each of 10 tissue culture dishes (35 x 10 mm) (Falcon Plastics Company, Oxnard, Calif.). Plates were incubated at 37° in a 10% CO₂ atmosphere with 100% humidity for 7 days. After that period of incubation, discrete colonies of cells were found, as well as small clusters of cells. Evidence provided previously (4) indicates that such cells are macrophages.
Identification marks on plates were replaced by a code to ensure objectivity of the colony counts. Only groups of 25 cells or more arranged in a colony configuration were counted. In general, the greater the colony count the larger were the colonies, suggesting that an increased number of macrophage precursors was associated with an increased rate of proliferation.

*M. bovis* strain BCG obtained from the Research Foundation, Chicago, Ill., was used. The vaccine, composed of viable organisms without preservative, was prepared by reconstituting ampuls containing 15 mg of dry powder with 1.5 ml of sterile water. Left hind legs of mice received s.c. injections of 0.1 ml of solution containing 1 mg, wet weight, equivalent (32 × 10⁶ viable units). In tumor-bearing mice, inoculations were in the region of but not into the tumors. Appropriate controls received the same volume of 0.9% NaCl solution in a similar location.

Experiment 1 was repeated 5 times; Experiments 2 and 3 were repeated 6 times. Data are expressed as mean colony counts. They were analyzed according to Student's *t* test with a level of significance of 0.01.

RESULTS AND DISCUSSION

Bone marrow cells from normal mice obtained 2, 5, or 7 days following BCG inoculation produced a significantly greater number of macrophage colonies than did those from similar animals that were recipients of 0.9% NaCl solution (Table 1). At 14 and 28 days after injection, the number of colonies from the marrow cells of BCG-injected animals was not significantly greater than from the normal controls. Bone marrow cells from BCG-inoculated animals with growing tumors produced a significantly greater number of macrophage colonies than did marrow cells from non-tumor-bearing mice given injections of BCG or 0.9% NaCl solution, or from mice that had a growing tumor but received no BCG (Table 2). During that period when BCG or tumor was effective, combination of the two intensified stimulation. At the time marrow cells either from animals with a growing tumor or from non-tumor-bearing BCG-treated mice failed to produce a greater number of macrophage colonies, the combination of tumor growth and BCG exposure resulted in a significant augmentation of macrophage production (Table 2 Experiment 3). An explanation for this synergistic effect is not available at present. The findings are in keeping with the increasing number of observations from experimental models that indicate that BCG seems to be more effective when administered in conjunction with tumor cells, and they also relate to those of Stjernswärd (13) who observed, by hemolytic plaque test, that BCG increases the number of antibody-producing cells. This may be accomplished by BCG stimulation of pluripotential stem cells in bone marrow to produce more macrophages, allowing for a greater uptake and processing of antigen, with a resultant increase in number of im-

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<th>Experiment</th>
<th>Days post-BCG</th>
<th>Days of tumor growth</th>
<th>No. of repetitions</th>
<th>Macrophage colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>5</td>
<td>5 (50 plates)</td>
<td>42.5 ± 11.4a</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>14</td>
<td>6 (60 plates)</td>
<td>54.1 ± 15.6</td>
</tr>
<tr>
<td>3</td>
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<td>21</td>
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* Mean ± S.D.
* N.S., not significant.
munologically competent cells. A variety of other mechanisms have been proposed to explain how macrophages participate in the host-immune response against its tumor. If those cells play a favorable role in that regard, it would seem that the benefit of BCG administration is related to augmentation of their production. Whether other non-specific immunostimulating agents (such as Corynebacterium parvum) similarly stimulate macrophages is under investigation.

ACKNOWLEDGMENTS

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REFERENCES

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