Macrophage Production by the Bone Marrow of Tumor-bearing Mice

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SUMMARY

Macrophage colony production in vitro by bone marrow cells was studied at intervals after the implant of a syngeneic mammary tumor into C3H female mice. Between 4 days and 2 weeks of progressive tumor growth, there was a significantly increased production of macrophage colonies from the tumor-bearing animals when compared with three types of controls. After 2 weeks, the colony production returned to normal. The implications of this relative to the response of the host to tumor are discussed.

INTRODUCTION

Evidence from experimental and human tumors indicates that the host is capable of mounting an immune response against a malignant process (17). That response is cellular, of the delayed hypersensitivity type, and/or humoral with production of antibodies that may cause enhancement of tumor growth (20).

Emphasis has been directed toward the role of the lymphocyte in the cellular aspect of the response. The macrophage has received scant attention despite increasing evidence that it plays a critical role in initiating and maintaining immune reactions. Feldmann and Palmer (16) have demonstrated that the macrophage is essential for initiating a delayed hypersensitivity response if the antigen is above a critical size. The increased "immunogenicity" of macromolecules bound to the surface of macrophages has also been demonstrated (12, 38). There is morphological evidence from electron microscopic studies of macrophage-lymphocyte interaction at the onset of an immune response (37). In general, it is accepted that macrophages process antigen for reaction with lymphocytes (1). Further emphasis on the critical role played by the macrophage is afforded by reports concerning restoration of the tuberculin reaction in experimental animals the delayed hypersensitivity response of which had been abrogated by prior radiotherapy (41). Peritoneal macrophages from a syngeneic, nonsensitized animal were capable of restoring the response. An obligatory role for the macrophage has also been demonstrated in the lymphocyte response to phytohemagglutinin (27). The macrophage also plays a part in maintaining the balance between cellular immunity and immunoglobulin-mediated enhancement by clearing excess antigen (40) or antibody-antigen complexes (19) from the circulation.

In experimental animals, the natural resistance to the development of tumors appears to parallel reticuloendothelial phagocytic activity (34). There is an increase in the reticuloendothelial phagocytic activity during the growth of transplanted syngeneic tumors (6, 24, 34) which is probably related to cellular factors rather than changes in opsonin levels (24). Coincident with this increased activity, there is a more rapid clearance of tumor cells from the circulation by hepatic sinusoidal macrophages (13). A heightened resistance to tumor induction has been noted in animals exhibiting a graft-versus-host reaction. This has been ascribed to the intense reticuloendothelial activation that occurs in that situation (14). In addition it has been shown that macrophages from immune animals have a specific action in the destruction of tumor cells (15), which is brought about by membrane contact (25).

In man a relationship between the degree of sinus histiocytosis of nodes draining a cancer and prognosis has been reported repeatedly (3, 4). A correlation between tumor size and clearance of colloidal particles from the blood by hepatic sinusoidal macrophages has been demonstrated (28). The effect of treatment on this parameter bore a relationship to the ultimate course of the disease (29). Finally, the appearance of large mononuclear cells at skin windows covered with cryostat sections of tumors has been shown to correlate with morphological evidence of host resistance to cancer and prognosis (5).

There seems to be little doubt that macrophages derive from rapidly dividing precursors in bone marrow (7, 26, 39, 42). In 1966 Bradley and Metcalf (8) described a method of culturing bone marrow cells in a semisolid medium. A modification of this technique has been used to study macrophage production by bone marrow cells from tumor-bearing mice.

MATERIALS AND METHODS

C3HeB/FeJ female mice, 8 to 12 weeks old, were used in all experiments. A spontaneous C3H mammary tumor maintained by serial implantation was used. Mice were inoculated with 1-mm plugs s.c. on the abdomen. Groups of normal mice,
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either sham operated or implanted with fragments of kidney tissue from mice of the same strain, and untreated animals served as controls. At intervals of 1 to 21 days after tumor implant, sham operation, or kidney implant, mice were sacrificed by cervical dislocation. The animals were immersed in antiseptic solution and one femur was removed by sterile techniques from each of 3 mice in each group. Marrow cells were harvested by drawing 5 ml of half-strength Solution A (see "Addendum") through the medullary cavity. An aliquot of the pooled marrow cell suspension was counted and was further diluted so that there were $10^6$ cells/ml. From the counts obtained it was possible to determine the total number of nucleated cells per femur (10) with an error of approximately 30%. One-tenth ml ($1 \times 10^5$ cells) was added to each of 10 tissue culture dishes (35 x 10 mm) (Falcon Plastics Co., Oxnard, Calif.) containing 0.64 ml methylcellulose solution (Solution B; see "Addendum") and 0.15 ml horse serum. The plates were incubated at 37° in a 10% CO₂ atmosphere with 100% humidity for 7 days. Studies had shown that after 7 days of incubation discrete colonies of 25 or more cells were always found in addition to clusters of fewer cells (Fig. 1). The cells when examined under high-power microscopy resembled macrophages (Fig. 2).

Identification marks on plates were replaced by a code so that the colony counts were objectively obtained. Only groups of 25 cells or more arranged in a colony configuration (Fig. 1) were counted. Clusters of cells were not recorded, nor was any attempt made to score the number of cells per colony. In general, it was found that the greater the colony count the larger were the colonies, suggesting that an increased number of proliferating macrophage precursors was associated with an increased rate of proliferation.

Data are expressed as mean colony counts per plate and as colony-forming cells per femur. They were analyzed according to the Student t test with a level of significance of 0.05.

RESULTS

Bone marrow from animals with a tumor present for 4 to 6 or 7 to 10 days produced a significantly greater number of macrophage colonies than did any of the 3 control groups. These differences were present whether data were expressed as colonies per plate (Table 1) or as colony-forming cells per femur (Table 2). When a tumor was present from 1 to 3 (with 1 exception) or for 14 to 21 days, however, colony counts from tumor-bearing animals were no greater than were those from controls.

DISCUSSION

The fact that findings were similar whether results were expressed as colonies per plate or as colony-forming cells per femur suggests that there is a qualitative change in the bone marrow of tumor-bearing mice that leads to an increased production of macrophages in vitro.

Bone marrow contains pluripotential stem cells that are capable of giving rise to all cell types found in peripheral blood (33). This has been demonstrated with an in vivo-irradiated mouse system. Marrow cells injected into such animals form colonies within the spleen, among which can be recognized members of all hemopoietic cell lines (2). The cells that proliferate in vitro in semisolid media are distinct and probably represent primitive members of the myeloblast series.

### RESULTS

<table>
<thead>
<tr>
<th>Days tumor was present</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor (colonies/10⁵ marrow cells)</td>
<td>32 ± 7.6⁴</td>
<td>49 ± 5.0</td>
<td>47 ± 8.8</td>
<td>90 ± 30.0</td>
<td>43 ± 4.5</td>
<td>114 ± 14.3</td>
<td>161 ± 40.0</td>
<td>106 ± 14.0</td>
<td>107 ± 9.1</td>
<td>17 ± 10.2</td>
<td>74 ± 6.3</td>
<td>90 ± 6.4</td>
<td>113 ± 8.8</td>
<td>72 ± 10.3</td>
<td>55 ± 21.5</td>
<td>24 ± 8.2</td>
<td>28 ± 10.3</td>
<td>31 ± 8.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (colonies/10⁵ marrow cells)</td>
<td>28 ± 5.5</td>
<td>54 ± 4.9</td>
<td>60 ± 8.0⁴</td>
<td>143 ± 17.5</td>
<td>28 ± 4.7⁵</td>
<td>36 ± 7.9⁶</td>
<td>58 ± 15.0⁶</td>
<td>55 ± 8.3⁵</td>
<td>86 ± 8.0⁵</td>
<td>42 ± 6.3⁶</td>
<td>52 ± 12.0⁶</td>
<td>44 ± 9.8⁶</td>
<td>43 ± 7.4⁶</td>
<td>36 ± 6.6⁵</td>
<td>53 ± 11.3</td>
<td>20 ± 4.5</td>
<td>77 ± 12.3⁴</td>
<td>31 ± 8.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>28 ± 5.5</td>
<td>54 ± 4.9</td>
<td>60 ± 8.0⁴</td>
<td>143 ± 17.5</td>
<td>28 ± 4.7⁵</td>
<td>36 ± 7.9⁶</td>
<td>58 ± 15.0⁶</td>
<td>55 ± 8.3⁵</td>
<td>86 ± 8.0⁵</td>
<td>42 ± 6.3⁶</td>
<td>52 ± 12.0⁶</td>
<td>44 ± 9.8⁶</td>
<td>43 ± 7.4⁶</td>
<td>36 ± 6.6⁵</td>
<td>53 ± 11.3</td>
<td>20 ± 4.5</td>
<td>77 ± 12.3⁴</td>
<td>31 ± 8.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁴ Mean of 6 to 10 plates ± S.D.
⁵ Lower than tumor $p < 0.05$.
⁶ Lower than tumor $p < 0.01$.

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Table 1

Macrophage colony production by 10⁵ marrow cells during tumor growth

Each line represents a separate experiment done at a separate time, i.e., 17 experiments.

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Table 2
Macrophage colony production by marrow cells per femur during tumor growth

<table>
<thead>
<tr>
<th>Days tumor was present</th>
<th>Tumor (colonies/femur x 10^3)</th>
<th>Controls (colonies/femur x 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Trauma</td>
</tr>
<tr>
<td>1</td>
<td>2.49 ± 0.6^a</td>
<td>2.62 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>3.85 ± 0.4</td>
<td>4.90 ± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>3.62 ± 0.7</td>
<td>3.38 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>5.13 ± 1.7</td>
<td>4.76 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>3.01 ± 0.3</td>
<td>2.48 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>9.70 ± 1.2</td>
<td>3.6 ± 0.8^c</td>
</tr>
<tr>
<td>4</td>
<td>8.06 ± 2.0</td>
<td>4.74 ± 1.2^c</td>
</tr>
<tr>
<td>4</td>
<td>5.99 ± 0.8</td>
<td>4.46 ± 0.7^c</td>
</tr>
<tr>
<td>6</td>
<td>10.10 ± 0.9</td>
<td>5.70 ± 0.5^c</td>
</tr>
<tr>
<td>7</td>
<td>7.89 ± 0.7</td>
<td>3.29 ± 0.5^c</td>
</tr>
<tr>
<td>7</td>
<td>8.24 ± 0.7</td>
<td>4.24 ± 1.0^c</td>
</tr>
<tr>
<td>7</td>
<td>6.31 ± 0.4</td>
<td>3.34 ± 0.7^c</td>
</tr>
<tr>
<td>10</td>
<td>6.85 ± 0.5</td>
<td>5.86 ± 0.9</td>
</tr>
<tr>
<td>10</td>
<td>7.50 ± 1.1</td>
<td>3.16 ± 0.6^c</td>
</tr>
<tr>
<td>14</td>
<td>4.05 ± 1.6</td>
<td>4.51 ± 1.0</td>
</tr>
<tr>
<td>14</td>
<td>2.43 ± 0.8</td>
<td>2.06 ± 0.5</td>
</tr>
<tr>
<td>21</td>
<td>2.32 ± 0.9</td>
<td>1.90 ± 0.5</td>
</tr>
</tbody>
</table>

^a Mean of 6 to 10 plates ± S.D.
^b Lower than tumor p < 0.05.
^c Lower than tumor p < 0.01.

The rapid return to normal levels of macrophage production in spite of increasing tumor growth provokes speculation. There may be only a limited supply of macrophage precursors capable of responding so that exhaustion soon occurs. Alternatively, the rapid accumulation of mature macrophages in the marrow under increasing stimulus may crowd out precursor cells which would correspondingly be plated in fewer numbers. Finally, a negative feedback may be operating whereby mature macrophages inhibit the production of new colonies by immature cells. Such a mechanism is thought to operate in the control of granulopoiesis (23, 36).

How the bone marrow response to tumor reported here may favor the host is also speculative. As a result of increased production a larger number of macrophages are available to carry out the following: (a) direct destruction of tumor cells relating to macrophage ability to recognize cells with abnormal growth characteristics (21); (b) augmentation of specific cell-mediated immunity by the increased uptake and processing of antigen; (c) inhibition of enhancing antibody production by the clearance of antigen-antibody complexes; (d) prevention of metastases by increased clearance of circulating malignant cells in the hepatic and splenic sinusoids.

It is possible that many agents that nonspecifically augment the host resistance to cancer, such as Bacillus Calmette-Guérin (34), Corynebacterium parvum (43), Freund's adjuvant (22), and estrogen (29), manifest their activity by the same mechanisms responsible for the response of macrophage precursors to the presence of a tumor.

The apparent exhaustion of this response after 2 to 3 weeks by which time the tumor is approximately 3% the weight of the host (equivalent to 2 kg tumor in an adult human) may provide a further explanation for the continued growth of an antigenic tumor in an immunologically intact host.
ACKNOWLEDGMENTS

We thank Dr. Paul Chervenick of the Department of Medicine for helping us with the bone marrow cultures, as well as Miss Francine Jencka and Miss Elizabeth Saffer for their expert technical assistance.

ADDENDUM

Stock Solution A (2 X 1066; Quantities to Prepare 2 Liters)
CMRL 1066 (10 x; Grand Island Biological Co., Grand Island, N. Y.)
400 ml
Double-distilled, pyrogen-free water
1294 ml
Sodium pyruvate (100 x; Grand Island Biological)
50 ml
Nonessential amino acids (100 x; Grand Island Biological)
50 ml
Essential amino acids (100 x; Grand Island Biological)
40 ml
7.5% NaHCO3
120 ml
L-Serine, 21 mg/ml (General Biochemicals Corp., Chagrin Falls, Ohio)
1 ml
L-Glutamine, 200 mM (Difco Corp., Detroit, Mich.; 300 mM in 60 ml made up to 100 ml in water
20 ml
L-Asparagine, 10 mg/ml (General Biochemicals)
solution (Grand Island Biologicals)
8 ml
Penicillin (100 units) and streptomycin (100 units)
20 ml
Mineral and essential medium vitamins (100 x; Grand Island Biological)
20 ml
Vitamin B12, 1000 µg/ml (E. R. Squibb & Sons, Inc., New York, N. Y.)
2.5 ml

Stock Solution B (1.8% Methylcellulose)
10.8 g methylcellulose, 4000 centipoise (Fisher Scientific Co., Fairlawn, N. J.; USP) dissolved in 300 ml of boiling, distilled, pyrogen-free water
Mixed with 300 ml Solution A
Stirred for 3 days in cold room until cleared

REFERENCES


Fig. 1. Characteristic macrophage colonies after 7 days of incubation. X 12.
Fig. 2. A group of cells from a 7-day colony. X 1200.
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