Endotoxin Enhancement of Plasma Cell Tumor Development in Mice Given Injections of Mineral Oil

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SUMMARY

Bacterial endotoxins administered to BALB/c mice given i.p. mineral oil cause an increased incidence of plasma cell tumors, compared with mice given either oil or antigens alone, or oil plus antigen other than endotoxin. Endotoxin in ng doses was more effective than in μg doses.

INTRODUCTION

Bacterial flora play a role in the development of PCT in the BALB/c mouse given oil injections (7, 10, 12). PCT does not develop at sites of MO injection other than the peritoneum (13). Germ-free BALB/c mice given i.p. MO form reticulosarcomas rather than PCT (7). Bacterial endotoxins are excellent candidates for an intermediate role in the production of PCT after oil injection. Globulins produced by several PCT’s cross-react with enteric antigens, suggesting prior exposure of precursor cells to these antigens (10). This paper describes a remarkable increase in the PCT formation when ng amounts of bacterial endotoxins are administered to mice given oil injections.

MATERIALS AND METHODS

Two separate experiments were done utilizing BALB/c female mice (Texas Inbred Mice Co., Houston, Texas, and Mammalian Genetics and Animal Production Section, Drug Research and Development, National Cancer Institute, Bethesda, Md.). The animals from the different suppliers were not mixed. The mice were given food and water ad libitum. At 2, 4, and 6 months of age, 0.5 ml of Primol D (Exxon Corporation, New York, N. Y.) was injected i.p. Within 24 hr of the 1st oil injection, groups of mice were given 1 of 4 antigens i.p. in a volume of 0.1 ml of 0.15 M NaCl. The appropriate dose of antigen was then given once a week thereafter for the course of the experiment. Mice given no oil injection were given antigen injections on the same schedule.

RESULTS

In the initial experiment done with BALB/c mice supplied by Texas Inbred Mice Co., 5 of 11 animals treated with 5.0 ng E. coli endotoxin developed recognizable PCT between 6 and 7 months of age (Table 1). Tumor nodules ranging in size from 1 to 10 mm were found throughout the mesentery. Two of the animals had tumor nodules over 18 mm in diameter. Four of the mice with PCT developed 5 to 6 ml of bloody ascites. In all of the tumor-bearing mice, the ascitic fluid contained cells characteristic of PCT (13). Electron micrographs of the tumor cells were compared with MOPC 315, and established line of PCT. Both tumors contained a large, well-developed Golgi complex, endoplasmic reticulum, and the type A particles of Bernhard typical of mouse PCT (2). The tumors induced by endotoxin treatment secreted an abnormal IgA globulin that did not precipitate with either E. coli or S. typhimurium endotoxin.

Notes:
1 Supported by USPHS Grant CA 14194, Grant CA 12635 from the National Cancer Institute, and Grant RR 05589 from General Research Support Program Division of Research Resources.
2 Present address: Schering-Plough Corporation, Bloomfield, N. J.
3 To whom requests for reprints should be addressed, at 1919 Madison Ave., New York, N. Y. 10035.
4 The abbreviations used are: PCT, plasma cell tumor; MO, mineral oil; BSA, bovine serum albumin; SRBC, sheep red blood cells.

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All mice from control, 5 μg endotoxin-, SRBC-, and both BSA-treated groups failed to develop ascites except for 2 mice receiving 5 ng of BSA. These 2 animals at autopsy did not have recognizable PCT. Peritoneal granulomas from these groups were devoid of malignant PCT by the histological criteria of Potter and MacArdle (13). The titers of IgG to either endotoxin, SRBC, or BSA in the MO-treated mice were not significantly different from antigen-challenged mice not given injections of MO.

The primary cell tumors induced by endotoxin were easily transplanted. Injection of 2 × 10^6 peritoneal cells produced ascites tumor in 21 of 21 of the recipients of oil injections.

### Table 1

**Chronic Antigenic treatment and induction of PCT in MO-treated BALB/c mice (Texas inbred)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>6 mos.</th>
<th>7 mos.</th>
<th>Cumulative no. of mice with PCT at 8 mos. of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Primol D only)</td>
<td>0/15</td>
<td>0/12</td>
<td>0</td>
</tr>
<tr>
<td>5 ng BSA</td>
<td>0/15</td>
<td>2/15</td>
<td>2</td>
</tr>
<tr>
<td>5 μg BSA</td>
<td>0/15</td>
<td>0/13</td>
<td>0</td>
</tr>
<tr>
<td>5 ng E. coli endotoxin</td>
<td>2/11</td>
<td>3/9</td>
<td>5 (p &lt; 0.01)</td>
</tr>
<tr>
<td>5 μg E. coli endotoxin</td>
<td>0/15</td>
<td>0/14</td>
<td>0</td>
</tr>
<tr>
<td>SRBC</td>
<td>0/12</td>
<td>0/12</td>
<td>0</td>
</tr>
</tbody>
</table>

* No PCT present in animals that died prior to 6 months.

### Table 2

**Tumor development after inoculation of peritoneal cells from control and antigen-treated mice (Experiment 1)**

<table>
<thead>
<tr>
<th>Donor group treatment</th>
<th>No. of donors</th>
<th>Status</th>
<th>No. of recipients developing PCT/total no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Primol D only)</td>
<td>5</td>
<td>Tumor negative</td>
<td>0/21</td>
</tr>
<tr>
<td>5 ng BSA</td>
<td>1</td>
<td>Tumor negative</td>
<td>0/5</td>
</tr>
<tr>
<td>5 μg E. coli endotoxin</td>
<td>3</td>
<td>Tumor negative</td>
<td>0/12</td>
</tr>
<tr>
<td>5 ng E. coli endotoxin</td>
<td>2</td>
<td>Tumor negative</td>
<td>0/17</td>
</tr>
<tr>
<td>SRBC</td>
<td>1</td>
<td>Positive</td>
<td>21/21</td>
</tr>
</tbody>
</table>

Table 3 shows the microscopic diagnosis at autopsy of the mice in Experiment 2. All mice with a gross diagnosis of within 18 days. The 2nd generation of these tumors could be raised within the same time interval in mice not given oil. Peritoneal cells from mice given oil injections, or from SRBC-, BSA-MO-treated mice did not give PCT even after 2 months in mice given injections of MO (Table 2).

The results of a 2nd experiment designed to determine the effective dose range of E. coli and S. typhimurium endotoxin as well are shown in Table 3. Between 27 and 47% of mice treated with 0.1, 1.0, or 5.0 ng E. coli endotoxin developed PCT between 8 and 12 months of age, compared to 11.0% of mice given only MO. Although the mice treated with 1 ng E. coli endotoxin showed a considerable increase in tumorigenesis, the p value did not show a significant increase in contrast to mice treated with 0.1 and 5 ng, in which highly significant differences were established. When control and BSA-treated mice were pooled and compared with mice treated with oil and E. coli endotoxin, regardless of dose, a highly significant (p < 0.005) difference was established. Endotoxin administration stimulates clinically evident PCT in BALB/c mice given oil injections. S. typhimurium endotoxin enhanced PCT induction in animals treated with 5.0 ng/week (p < 0.005); it was ineffective at the lower dose of 0.1 ng. In both cases, injection of 5.0-μg amounts of the endotoxins did not increase PCT. Groups treated with 0.1 ng to 5.0 μg BSA also did not show increased tumorigenesis over mice treated with oil alone. At autopsy, or by histological examination, no evidence of PCT formation was found in groups not given oil, but given 0.15 M NaCl solution, and similar doses of BSA, E. coli, and S. typhimurium.

Although the basic result of PCT induction by ng amounts of endotoxin was obtained in both experiments, we did observe that there were differences in the time needed for the tumor to develop and in the quantity and characteristics of the PCT formed. PCT took somewhat longer to appear in the 2nd experiment. The amount of ascites fluid was extremely variable, between 0.5 and 5 ml. The tumor did not form in large nodules but was intermingled with granulomas. Transplanted PCT did not grow readily in mice given MO injections.

### Table 3

**Chronic antigenic treatment and induction of PCT in MO-treated BALB/c mice (NIH) (Experiment 2)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>8 mos.</th>
<th>12 mos.</th>
<th>% gross tumor incidence at autopsy</th>
<th>Microscopic diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO (Primol D)</td>
<td>0/30</td>
<td>3/27</td>
<td>11.0%</td>
<td>7/27</td>
</tr>
<tr>
<td>0.1 ng E. coli endotoxin</td>
<td>1/30</td>
<td>11/26</td>
<td>42.2% (p &lt; 0.025)</td>
<td>20/26 (p &lt; 0.005)</td>
</tr>
<tr>
<td>1 ng E. coli endotoxin</td>
<td>1/30</td>
<td>7/26</td>
<td>26.8%</td>
<td>14/26</td>
</tr>
<tr>
<td>5 ng E. coli endotoxin</td>
<td>1/30</td>
<td>13/29</td>
<td>44.8% (p &lt; 0.025)</td>
<td>13/29</td>
</tr>
<tr>
<td>5 μg E. coli endotoxin</td>
<td>0/30</td>
<td>8/25</td>
<td>28.4%</td>
<td>13/25</td>
</tr>
<tr>
<td>0.1 ng S. typhimurium endotoxin</td>
<td>0/27*</td>
<td>1/27</td>
<td>3.7%</td>
<td>11/27</td>
</tr>
<tr>
<td>5 ng S. typhimurium endotoxin</td>
<td>1/30</td>
<td>14/25</td>
<td>55.0% (p &lt; 0.005)</td>
<td>20/25 (p &lt; 0.005)</td>
</tr>
<tr>
<td>5 μg S. typhimurium endotoxin</td>
<td>1/30</td>
<td>2/29</td>
<td>6.9%</td>
<td>4/24</td>
</tr>
<tr>
<td>0.1 ng BSA</td>
<td>0/30</td>
<td>4/30</td>
<td>13.8%</td>
<td>12/30</td>
</tr>
<tr>
<td>1 ng BSA</td>
<td>0/21†</td>
<td>1/21</td>
<td>4.9%</td>
<td>8/21</td>
</tr>
<tr>
<td>5 μg BSA</td>
<td>0/24*</td>
<td>4/23</td>
<td>17.4%</td>
<td>11/23</td>
</tr>
</tbody>
</table>

* p values were determined by χ² test with Yates, correction between Primol D control and each treated group.

* Mice died prior to 3rd oil injection.
tumor showed evident tumor by histological examination. In addition, many mice showed foci of nascent tumor within granulomata. Since it is difficult to be certain that small, isolated infiltrates of large plasma cells represent tumor, these were disregarded. Table 3 indicates a positive diagnosis when unequivocal multiple foci of tumor cells were present. Because twice as many mice treated with MO alone showed microscopic foci as showed gross tumor, the effect of treatment was somewhat blurred. Only mice treated with 0.1 ng E. coli endotoxin or 5 ng S. typhimurium endotoxin showed a significant difference from controls. However, when BSA-treated mice were pooled with controls and compared with a pool from all ng-dose-treated mice, the microscopic incidence of tumor was significant at $p < 0.025$. Histologically, the cells were typical of PCT (13). Electron micrographs confirmed the cells as PCT, although they did not contain heavy concentrations of virus particles, in contrast to the tumor cells from the 1st experiment.

**DISCUSSION**

Induction of PCT depends upon the interaction of several factors: a favorable genotype, a bacterial flora, virus activation, and the presence of an inflammatory agent in the peritoneum (7–14). Our results clearly indicate that, depending upon experimental conditions, ng amounts of exogenous bacterial endotoxin will enhance the rate of PCT induction (Experiment 1), or increase the percentage of mice with gross PCT by 12 months of age (Experiment 2). Activation of C-type RNA viruses in the B-cell line is a potent factor in determining PCT development (12). During tumorigenesis, several viruses may be activated (9, 12, 14). This variable, as well as the presence of bacterial antigens, can control the rate of tumor development. The variability in the response to MO alone, and to injected antigens observed in our experiments, can be attributed to the inherent differences in viral and bacterial constituents between inbred mice from different breeding farms and between individual animals.

Chronic administration of growth hormone and testosterone also enhance tumorigenicity of MO in BALB/c mice (5). It is not known whether these hormonal treatments or the synergistic viruses affect the endotoxin concentration in the blood of oil-treated animals.

Endotoxin can play many roles in influencing the neoplastic process, from its well-known effects on proliferation of B-cells (4, 15) to the production of PCT growth factors (3, 8). They may also augment viral activation. Studies are now in progress to explore the unexpected dose effect and specificity of endotoxin.

The lack of PCT development when $\mu$g rather than ng doses were used cannot be explained. But at least one other study in our laboratory confirms the effectiveness of ng rather than $\mu$g doses of endotoxin. Such studies (1) on recipients of PCT transplants show an inhibition of tumor growth with ng doses; higher doses were less effective.

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**REFERENCES**

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