Transplantation of Primary Plasma Cell Tumor without 2,6,10,14-Tetramethylpentadecane (Pristane) Treatment of the Hosts

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ABSTRACT

Small inocula of primary plasma tumor cells which do not transplant i.p. unless recipients are conditioned with pristane do so readily when recipients are given i.p. injections of a peritoneal exudate induced by pristane inoculation, but free of pristane.

INTRODUCTION

The injection of mineral oil or any of its constituents, e.g., 2, 6, 10, 14-tetramethylpentadecane (pristane), into the peritoneal cavity of BALB/c mice can induce plasmacytoma (PCT) after a latent period of 5 to 10 months (1, 7). Transplantation of a limited number of such nascent tumor cells i.p. almost never results in tumor growth. However, successful transplantation is achieved if recipient mice are conditioned by i.p. injections of mineral oil or pristane at least 3 days before i.p. inoculation of ascitic tumor cells (3, 8). We now report that PPCT, which does not transplant, does so readily when recipients are given i.p. injections of a peritoneal exudate engendered by, but free of, pristane.

MATERIALS AND METHODS

Primary Plasma Tumor Cells. Female BALB/c mice (Simon Laboratories, Gilroy, Calif.) received 3 i.p. injections of 0.5 ml of pristane at 2, 4, and 6 months of age. Ascitic fluid was collected 12 to 16 months after pristane injection, and primary plasma tumor cell suspensions were adjusted to $1 \times 10^8$ tumor cells/ml. An 0.1-ml dose of this suspension was administered i.p. to randomized recipient mice assigned to different treatment groups. PPCT was diagnosed in ascitic fluid by the presence of many large hyperchromatic plasma tumor cells.

PIPE. Female BALB/c mice, 6 to 8 weeks old, were given injections of pristane (0.5 ml i.p.). Three days or 2 months later, 3 ml of sterile 0.85% NaCl solution were injected i.p. After gentle massage, their shaved and Betadine-scrubbed abdomens were incised and penitoneal fluid was collected. Bloody fluids were discarded. The combined penitoneal fluids were centrifuged at 20 x g for 3 min. The top layer containing free pristane and pristane-filled macrophages was removed. The remaining pellet, combined with supernatant, was designated PIPE. The cellular component of PIPE contained few oil-filled macrophages (since most of them had been removed by flotation). Lymphocytes, granulocytes, and plasma cells could also be identified. The total cell counts were about 1 to 2 x $10^9$ cells/ml. Similar preparations were made from CBA/J mice, a strain that does not develop plasmacytomas after pristane treatment. PIPE treatments were given to BALB/c mice as i.p. injections of 0.5 ml each, either as a single injection given 3 days before primary tumor inoculation or as 3 injections given for 3 successive days prior to tumor inoculation. Some recipients also received 0.1 ml 0.85% NaCl solution i.p., 1 ng LPS, or 5 $\mu$g LPS at the time of PIPE treatment. *Escherichia coli* LPS 055:B5 was obtained from Difco Laboratories, Detroit, Mich.

Fractionation of PIPE. PIPE samples were centrifuged at 150 x g for 5 min, and 2 fractions, supernatant and cell pellet, were separated. The recombination of the 2 layers was designated as reconstituted PIPE.

Pristane. Pristane was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. $[^3H]$Pristane, prepared by the Wilzbek exchange method, was dissolved in petroleum ether, extracted with concentrated H$_2$SO$_4$, neutralized with 1 M NaOH, and washed with distilled water. The $[^3H]$pristane and the unlabeled pristane were chromatographed on thin-layer Silica G (1 mm thick; 20- x 20-cm plates); solvent system was methanol:chloroform, 1:3; R$_f$ 0.5. Material used as a pristane tracer was purified by chromatography in this system.

RESULTS

Pristane-free PIPE. In order to be certain that PIPE did not contain pristane complexed with proteins so that it could not be separated by centrifugation from the aqueous phase, 2 methods were used. (a) PIPE samples were extracted with petroleum ether. Such treatment readily recovered added pristane (80 to 100% recovery), but gave only a trace of lipid residue when PIPE was extracted. The small residue was not pristane, since it was removed from petroleum ether by extraction with concentrated H$_2$SO$_4$ which does not remove pristane. With this method, as little as 1 $\mu$g pristane could be detected by weighing the residue; no significant amount was found in PIPE. (b) $[^3H]$Pristane (0.5 ml) containing $1 \times 10^7$ cpm was inoculated into BALB/c mice. PIPE prepared 3 days or 2 months later contained no radioactivity. The recovered radioactive material was detected in the top layer which was discarded. No radioactivity was found in either blood or urine of pristane-inoculated mice. With this method, as little as 1 $\mu$g pristane could be detected. It was established that, to condition an animal to grow PPCT, it is necessary to use at least 100 $\mu$g of pristane.

Table 1 compares the tumor development in mice given inoculations of $1 \times 10^4$ PPCT cells and conditioned by either
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Table 1
PPCT transplantation aided by PIPE

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of tumors diagnosed/total no. of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>35 days</td>
</tr>
<tr>
<td>1</td>
<td>0.5 ml pristane</td>
<td>0/20</td>
</tr>
<tr>
<td>2</td>
<td>0.5 ml PIPEa</td>
<td>2/20</td>
</tr>
<tr>
<td>3</td>
<td>1.5 ml PIPEe</td>
<td>5/10</td>
</tr>
<tr>
<td>4</td>
<td>0.5 ml PIPEc</td>
<td>0/5</td>
</tr>
<tr>
<td>5</td>
<td>0.5 ml reconstituted PIPEc</td>
<td>0/10</td>
</tr>
<tr>
<td>6</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>7</td>
<td>1.5 ml supernatant, PIPEc</td>
<td>0/10</td>
</tr>
<tr>
<td>8</td>
<td>1.5 ml pellet, PIPEc</td>
<td>0/10</td>
</tr>
</tbody>
</table>

a PIPEe, PIPE prepared from BALB/c mice given inoculations of pristane 3 days prior to collection.

b Mice were not given i.p. injections of PPCT on Day 4.

c NS, not significant.

The growth of primary plasma tumor cells in PIPE-conditioned mice is reproducible. Significant transplantation occurred in all of our experiments. Unfortunately, in spite of all of our efforts to identify the active fraction of PIPE, we were unable to determine whether activity resides in the cellular or in the soluble fraction. In addition, the reconstituted PIPE also failed to condition the mice to grow plasmacytomas. This could be due to either the instability of the factor or the very low activity of either pellet or supernatant.

Since we have reported that small quantities of LPS given pristane or PIPE collected 3 days after pristane inoculation. The experiment was repeated 4 times and similar results were obtained. Tumor diagnosis was based on the presence of large malignant plasmacytoma cells in bloody ascitic fluid, the gross findings of tumor at autopsy, and the capacity of the ascitic fluid and ability of these cells to develop PCT in pristane-conditioned mice.

Pristane or PIPE collected 3 days after pristane inoculation. The experiment was repeated 4 times and similar results were obtained. Tumor diagnosis was based on the presence of large malignant plasmacytoma cells in bloody ascitic fluid, the gross findings of tumor at autopsy, and the capacity of the ascitic fluid and ability of these cells to develop PCT in pristane-conditioned mice. Histological examination showed the tumors to be typical plasmacytomas. Animals given only small inocula of primary tumor cells (1 x 10⁶ cells/mouse) failed to show tumor growth even after 100 days. In contrast, successful transplantation was seen in many mice given 3 PIPE treatments. The PIPE conditioning activity was dose dependent. At high doses of PIPE, no significant differences were seen between pristane and PIPE conditioning capacity. No tumors developed in mice when given PIPE without PPCT. Neither reconstituted nor fractionated PIPE (supernatant or pellet) allowed transplantation of small inocula of PPCT.

The growth of PPCT in mice receiving PIPE collected 2 months after i.p. inoculation of pristane is presented in Table 2. Tumor development in mice treated with PIPE was slower than that seen in mice given pretreatment with pristane. A single inoculation of PIPE was ineffective. LPS or sham injections given along with PIPE did not increase the PPCT outgrowth compared with the effect given by PIPE alone (not shown in Table 2). No tumor developed when PIPE was given without primary plasma tumor cells. Sham, 0.85% NaCl solution, or LPS administration for 3 days prior to PPCT inoculation had no effect on tumor outgrowth. Reconstituted and fractionated PIPE also failed to condition the animals to take PPCT.

The strain specificity of PIPE was also tested. PIPE prepared from pristane-injected CBA/J mice did not allow the recipient mice to grow PPCT. Animals treated with peritoneal washings collected from 0.85% NaCl solution-inoculated normal BALB/c or CBA/J mice also failed to show tumor growth even after 100 days. When PIPE obtained from pristane-injected CBA/J mice was administered along with 1 ng LPS or sham injections daily for 3 days prior to tumor cell challenge, 1 of 5 of the mice developed PPCT in each of the 2 groups. These few successful transplants may indicate slight activity and require further study (Table 3).

DISCUSSION

The growth of primary plasma tumor cells in PIPE-conditioned mice is reproducible. Significant transplantation occurred in all of our experiments. Unfortunately, in spite of all of our efforts to identify the active fraction of PIPE, we were unable to determine whether activity resides in the cellular or in the soluble fraction. In addition, the reconstituted PIPE also failed to condition the mice to grow plasmacytomas. This could be due to either the instability of the factor or the very low activity of either pellet or supernatant.

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chronically for months to pristane-injected mice increased PCT development (2) and that peritoneal fluid collected from pristane-injected mice contained increased LPS (6), we also tried to see the effect of ng amounts of bacterial LPS added to PIPE.

We have suggested that mineral oil or pristane injections caused a leak of LPS from the gut and that this LPS would play a role in the pathogenesis of PCT. Apparently, it plays no role in acute enhancement of transplantation of primary tumor cells. Our current working hypothesis on the nature of PIPE is that pristane has an immunosuppressant action probably operating through suppressor cells. It was reported that pristane had immunosuppressive effects (4, 9). We have recently shown that s.c. pristane stimulates the outgrowth of a small s.c. inoculum of MOPC-315 (5), an established transplantable PCT line. Locally, pristane produces an intense inflammatory reaction which may give rise to suppressor cell outgrowth. Both stress and inflammatory reaction were ruled out in those studies.

Because PIPE may constitute a model for situations favorable to the development of human myeloma, the identification of the active factor is of importance.

Our current effort involves studying PIPE in a colony-stimulating system which requires PCT cells, PIPE, and LPS for colony formation in soft agar. With this more rapid system, we expect to study the mechanism and the nature of PIPE without the stability problems which have prevented efforts at separation of PIPE in the in vivo system.

REFERENCES

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