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 plasma cell tumor (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- sen 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 $1 \times 10^6$ 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 larger hyperplastic 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 peritoneal fluid was collected. Bloody fluids were discarded. The combined peritoneal 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 $10^7$ 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 μg 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.

[$^3$H]Pristane, prepared by the Wilzbach 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 [$^3$H]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 10 μl pristane could be detected by weighing the residue; no significant amount was found in PIPE. (b) [$^3$H]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 μg pristane could be detected. It was established that, to condition an animal to grow PPCT, it is necessary to use at least 100 μl of pristane.

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

Since we have reported that small quantities of LPS given

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>35 days</th>
<th>45 days</th>
<th>55 days</th>
<th>100 days</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5 ml pristane</td>
<td>0/20</td>
<td>0/20</td>
<td>0/20</td>
<td>0/20</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.5 ml PIPEa</td>
<td>0/20</td>
<td>2/20</td>
<td>5/20</td>
<td>5/20</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>1.5 ml PIPEb</td>
<td>5/10</td>
<td>7/10</td>
<td>9/10</td>
<td>9/10</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>0.5 ml PIPEc</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>NS</td>
</tr>
<tr>
<td>5</td>
<td>0.5 ml reconstituted PIPEb</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>1.5 ml supernatant, PIPEa</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>NS</td>
</tr>
<tr>
<td>7</td>
<td>1.5 ml pellet, PIPEa</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of tumors diagnosed/total no. of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5 ml pristane</td>
<td>0/65/65/65</td>
</tr>
<tr>
<td>2</td>
<td>0.5 ml PIPEb</td>
<td>26/28 (93)%/26/28 (93)%/26/28 (93)%</td>
</tr>
</tbody>
</table>
| 3     | 0.5 ml PIPEc | 11/21 (52%)/15/21 (71%)/15/21 (71%)
| 4     | 0.5 ml reconstituted PIPEb | 0/15 | 0/15 | 0/15 | 0/15 |
| 5     | LPS (1 ng, 5 μg) | 0/15 | 0/15 | 0/15 | 0/15 |
| 6     | Sham | 0/15 | 0/15 | 0/15 | 0/15 |
| 7     | 0.85% NaCl solution | 0/15 | 0/15 | 0/15 | 0/15 |
| 8     | 0.5 ml PIPEa | 0/15 | 0/15 | 0/15 | 0/15 |
| 9     | 0.5 ml reconstituted PIPEa | 0/18 | 0/18 | 0/18 | 0/18 |
| 10    | 1.5 ml supernatant, PIPEa | 0/18 | 0/18 | 0/18 | 0/18 |
| 11    | 1.5 ml pellet, PIPEa | 0/18 | 0/18 | 0/18 | 0/18 |

**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.

Since we have reported that small quantities of LPS given...
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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