Effect of Subcutaneously Administered 2,6,10,14-Tetramethylpentadecane on Plasmacytoma Growth

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ABSTRACT

2,6,10,14-Tetramethylpentadecane (pristane), s.c. injected simultaneously with plasmacytoma inocula, enhances the transplantability of tumor cells. The effect is dose and time dependent. The enhancement is shown only by plasmacytoma (MOPC-315 and MPC-11) and not by the murine lymphosarcoma and chondrosarcoma tested. Various mechanisms, such as stress and depression of humoral and cellular immunity, have been considered.

INTRODUCTION

Anderson and Potter (2) showed that i.p. injection of 2,6,10,14-tetramethylpentadecane (pristane) into BALB/c mice would induce PCT development after a latent period of 6 to 10 months. Small inocula of such primary plasma tumor cells would not transplant when injected i.p. into syngeneic mice. The i.p. administration of pristane readily allowed successful transplantation of primary plasma tumor cells (6, 19).

We now report that pristane injected s.c. enhanced the transplantability of a small s.c. inoculum of transplantable plasmacytomas. The effect was limited to plasmacytomas; no effect of pristane was seen on murine lymphosarcoma or chondrosarcoma.

In our studies concerning mechanisms involved in the s.c. pristane effect, several possibilities, such as stress and depression of cellular or humoral immunity, were considered.

MATERIALS AND METHODS

Tumors

MOPC-315. The source and maintenance of MOPC-315 have been described (3). Female BALB/c mice, 6 to 8 weeks old, were inoculated in the right paravertebral area with a suspension of 1 × 10⁶ MOPC-315 cells. This inoculum produced palpable tumors in about 50% of otherwise untreated mice by 30 days.

MPC-11. A plasmacytoma that produces a γG₂b paraprotein was obtained from Dr. M. Scharff (Albert Einstein College of Medicine, New York, N. Y.) and transplanted as described for MOPC-315.

P1798 Lymphosarcoma (Corticoid-sensitive and -resistant). A tumor induced in BALB/c mice with diethylishalbestrol-

cholesterol was transplanted according to the method described by Stevens et al. (26) with slight modifications. Inocula of 5 × 10⁵ cells s.c. produced palpable tumors in about 50% of the mice in 30 days.

DCII Chondrosarcoma. A spontaneous transplantable tumor which arose in ST/Eh mice was obtained and transplanted as described by Swarm (28). Mice inoculated with 1 × 10⁶ cells, prepared as described for MOPC-315, gave a 40% outgrowth in about 50 days.

Tumor cells were given alone or coincidentally with s.c. 2,6,10,14-tetramethylpentadecane (pristane; Aldrich Chemical Co., Milwaukee, Wis.). Pristane purity was established as described previously (18).

Some groups of mice received s.c. mineral oil, Primol 355 (Exxon Corp.), silicic acid (100 mesh; Mallinckrodt Chemical Works, St. Louis, Mo.), or surgical cotton (0.2-cm-diameter pellets) along with the tumor cells. Other groups received daily s.c. injections of ACTH (Acthar gel; Armour Pharmaceutical Co., Phoenix, Ariz.) in doses of 0.5 to 2.5 mg.

Preparation of Lymphocytes

Spleen Lymphocytes. Spleens were aseptically removed and minced. The splenocytes were extruded by gentle pressing in a glass homogenizer. Large aggregates and debris were removed by passing them through a stainless steel sieve. The remaining splenocyte suspension was washed twice and resuspended in Roswell Park Memorial Institute Tissue Culture Medium 1640. Lymphocytes were prepared from splenocyte suspensions using a Ficoll-Hypaque gradient (specific density, 1.076 to 1.080). Mice inoculated with 1 × 10⁶ cells, prepared as described for MOPC-315, gave a 40% outgrowth in about 50 days.

Mesenteric Lymph Node Lymphocytes. These were prepared using the same method as described for spleen lymphocytes, excluding the Ficoll-Hypaque step.

Lymphoid Response to Mitogens

Each microculture contained 2 × 10⁵ cells in 0.2 ml Roswell Park Memorial Institute Tissue Culture Medium 1640, supplemented with 10% heat-inactivated fetal calf serum. Cultures stimulated with 50 μg LPS or 10 μg PHA (Difco Laboratories, Detroit, Mich.) were incubated at 37°C for 48 to 72 hr in a humidified atmosphere of 5% CO₂ in air. LPS and PHA stimulatory effects were measured at 48 and 72 hr by adding 0.3 μCi [³H]thymidine (specific activity, 20 Ci/mmol) to each of 4 replicate cultures. The cultures were harvested 4 hr later using an automated multiple cell harvester (Rockefeller University, New York, N. Y.). Data were obtained at 48 and 72 hr, but for convenience only 48-hr data are presented. Conclusions from both time periods were identical. Results are expressed as the
average net counts of the 4 replicate cultures ± S.E., unless otherwise indicated. The net counts were obtained by subtracting the average cpm of the control unstimulated cultures from the cpm of the individual mitogen-stimulated cultures.

Hemagglutinin Titters

Mice were immunized by i.v. inoculation of SRBC at different times after tumor inoculation (1, 3, 7, 14, and 30 days). Four days later, blood was collected and tested for hemagglutinin. The greatest dilution giving unequivocal agglutination of SRBC after 24 hr of refrigeration was recorded as the titer of the serum.

Histology

Specimens from the site of pristane and PCT inoculation and from spleen, liver, adrenal, thymus, and tumor, when present, were taken for microscopic study at different times after PCT inoculation.

RESULTS

Enhancement of PCT Outgrowth by Pristane. Administration of 0.5 ml pristane s.c. at the site of tumor inoculation or at a distance had a highly significant effect on transplanted MOPC-315. Tumors appeared earlier and occurred in greater number in mice given s.c. pristane compared to animals which received only PCT. In contrast, 0.5 ml pristane injected i.p. had only a slight enhancing effect. The tumor take rate in the pristane plus PCT group varied from 80 to 100%, compared to 40 to 50% in the control group (PCT alone). Growth of MPC-11, another transplantable plasmacytoma line, was significantly enhanced by pristane administration at the same or opposite side as tumor inoculation. In contrast, murine chondrosarcoma and lymphosarcoma P1798 (corticoid-sensitive and -resistant) were unaffected by pristane treatment (Table 1).

Experiments were terminated at 30 days after tumor cell inoculation, since no increase in the number of tumor-bearing animals was observed after that time. Tumors tended to be larger in the mice treated with pristane plus PCT at the same or opposite side (data not shown).

Table 2 shows that doses of 0.5 to 0.1 ml pristane were effective in increasing the yield of MOPC-315 tumors at 30 days; doses below 0.1 ml were ineffective. The enhancing effect of pristane was seen only if the hydrocarbon was given at the time of tumor inoculation. If the pristane was given 3 days before or at various times after inoculation, no effect was noted.

Lack of Increased Outgrowth of MOPC-315 Tumors Caused by ACTH. Since pristane produced an active inflammatory response in s.c. tissues, we sought evidence that stress might alter tumor growth by affecting the hypothalamic-adrenal axis. If stress is involved (stimulation with ACTH), changes in thymus and adrenal weights and in blood leukocyte counts would be seen.

Table 3 shows that doses of 0.5, 1.0, or 2.5 mg ACTH gel do not cause increased transplantability of MOPC-315 tumors. On the contrary, the 2.5-mg dose inhibited the pristane effect, presumably by antiinflammatory action. ACTH treatment produced a highly significant increase in adrenal weight and decrease in thymus weight 7 days after beginning the treatment, but no significant changes in body or spleen weight were seen. Compared to normal control mice, animals inoculated with tumor cells (with and without pristane) or with pristane alone showed no differences in total body, adrenal, and thymus weights. However, a highly significant increase (p < 0.025) in spleen weight in animals given injections of pristane (alone or with PCT) was obtained 7 days after the beginning of the experiment (data not shown). Pristane injection (alone or with tumor cells) changed the total WBC counts of hosts in a manner different from that produced by PCT inoculation or ACTH treatment. Within 2 hr after injection, ACTH or MOPC-315 produced a significant drop in WBC counts, followed by a

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Type of tumor</th>
<th>Treatment</th>
<th>No. with tumors/total [%]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 MOPC-315</td>
<td>0.5 ml pristane i.p.</td>
<td>12/30</td>
<td>40</td>
<td>NS</td>
</tr>
<tr>
<td>2 MOPC-315</td>
<td>0.5 ml pristane s.c., same side</td>
<td>10/20</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>3 MOPC-315</td>
<td>0.5 ml pristane s.c., same side</td>
<td>12/24</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>4 MOPC-315</td>
<td>0.5 ml pristane s.c., same side</td>
<td>12/24</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>5 MOPC-315</td>
<td>0.5 ml pristane s.c., same side</td>
<td>12/24</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>6 P1798 CR</td>
<td>0.5 ml pristane s.c., same side</td>
<td>7/10</td>
<td>70</td>
<td>NS</td>
</tr>
<tr>
<td>7 P1798 CR</td>
<td>0.5 ml pristane s.c., same side</td>
<td>5/10</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>8 P1798 CS</td>
<td>0.5 ml pristane s.c., same side</td>
<td>6/10</td>
<td>60</td>
<td>NS</td>
</tr>
<tr>
<td>9 P1798 CS</td>
<td>0.5 ml pristane s.c., same side</td>
<td>3/10</td>
<td>30</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Diameter, >3 mm.

b NS, not significant. CR, corticoid-resistant. CS, corticoid-sensitive.

c Animals with tumors 50 days after tumor inoculation.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (ml)</th>
<th>Time relative to PCT inoculation</th>
<th>No. with tumors/total [%]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 MOPC-315</td>
<td>0.010</td>
<td>Same day</td>
<td>8/20</td>
<td>40</td>
</tr>
<tr>
<td>2 MOPC-315</td>
<td>0.030</td>
<td>Same day</td>
<td>10/24</td>
<td>42</td>
</tr>
<tr>
<td>3 MOPC-315</td>
<td>0.1</td>
<td>Same day</td>
<td>17/20</td>
<td>85</td>
</tr>
<tr>
<td>4 MOPC-315</td>
<td>0.3</td>
<td>Same day</td>
<td>19/20</td>
<td>95</td>
</tr>
<tr>
<td>5 MOPC-315</td>
<td>0.5</td>
<td>Same day</td>
<td>19/20</td>
<td>95</td>
</tr>
<tr>
<td>6 MOPC-315</td>
<td>0.5</td>
<td>3 days prior</td>
<td>13/20</td>
<td>65</td>
</tr>
<tr>
<td>7 MOPC-315</td>
<td>0.5</td>
<td>3 days after</td>
<td>8/20</td>
<td>40</td>
</tr>
<tr>
<td>8 MOPC-315</td>
<td>0.5</td>
<td>3 days after</td>
<td>6/20</td>
<td>30</td>
</tr>
<tr>
<td>9 MOPC-315</td>
<td>0.5</td>
<td>2 wk after</td>
<td>6/20</td>
<td>30</td>
</tr>
</tbody>
</table>

* NS, not significant.
recovery to almost normal levels within 3 hr. By 24 hr, WBC counts in these mice were significantly higher than in the normal untreated mice. In contrast, injection of pristane, whether given alone or with tumor cells, produced a sharp drop in WBC, which was still at nadir 3 hr after oil administration. At 24 hr, the WBC level reached normal values. A decrease in the leukocyte count in all 4 groups was recorded by Day 7, followed by normalization 7 days later.

**Changes in the Response of Splenocyte to PHA Stimulation Caused by Pristane.** To measure T-cell function in treated mice, we studied the ability of splenocytes to proliferate in response to PHA stimulation at different times after tumor inoculation, from latency period through progressive tumor growth (27). Chart 1 presents the results of splenocytes collected from 3 treatment groups (pristane alone, tumor-inoculated, and tumor-inoculated plus pristane), compared with normal control mice, to PHA stimulation, as determined by [3H]thymidine incorporation. A decrease in [3H]thymidine incorporation by 2 hr, followed by relative hyperreactivity at 24 and 72 hr, was recorded in all mice inoculated with tumor or pristane. By 7 days, the response to PHA started to decrease slightly and was maintained at lower levels thereafter. However, the differences between control and tumor-inoculated animals without palpable tumors were not significant. In contrast, when splenocytes from tumor-bearing animals were stimulated with PHA 21 days after tumor inoculation, thymidine incorporation was decreased (p < 0.001) whether or not pristane had been injected. The decrease was significantly greater (p < 0.025) in the pristane plus PCT group compared to mice given only PCT. Pristane treatment alone significantly decreased the [3H]thymidine incorporation in PHA-stimulated splenocytes at 21 days (p < 0.001). The response of normal splenocytes to PHA was inhibited by splenocytes from pristane-treated animals (with or without PCT).

No differences between treatment groups were noted when LPS was used as a mitogen. When splenocytes from animals without palpable tumors were stimulated with LPS (50 μg), no significant differences were found among any groups (normal control, pristane-treated, PCT-inoculated, or PCT plus pristane-inoculated). However, splenocytes from tumor-bearing mice showed a decreased response to LPS compared to normal animals. No significant differences between groups treated with pristane alone and PCT alone were found (Table 4). Similar results were obtained with lymphocytes from spleen or mesenteric lymph nodes stimulated with LPS.

**Lack of Depression of SRBC Antibody Formation Caused by Pristane.** Injection of pristane coincident with s.c. inoculation...
tion of MOPC-315 cells had no significant effect on the antibody titer against SRBC as long as the tumor did not develop. Similar results were obtained in groups of mice treated s.c. only with pristane. In contrast, tumor-bearing animals from either group, PCT alone or PCT plus pristane, had severely depressed responses to SRBC, but no significant differences between the 2 groups were observed (data not shown).

Histologic Examination. The site of pristane injection showed an intense inflammatory response. As early as 7 days after pristane treatment, oil-filled macrophages and polymorphonuclear cells had infiltrated the area. The response persisted for at least 30 days, at which time the response had progressed to an abscess-like appearance. Histological examination of the site of tumor injection revealed no changes that might explain the earlier appearance of tumors. No obvious inflammatory or vascular response at the site of tumor injection was noted beyond a minimal inflammatory response, which was the same whether or not pristane had been injected at a distant site. Histological examination of the adrenals of tumor-bearing animals showed no changes with respect to control animals, whether or not pristane had been administered. Specifically, no hyperplasia of a particular zone was evident.

DISCUSSION

Cancro and Potter (6) and Potter et al. (19) showed that pristane administered i.p. prior to tumor inoculation can condition the animals to accept transplants of small inocula of primary plasma tumor cells. Pristane i.p. injected can also abolish an immunity established to a particular PCT (20).

We found that s.c. administration of pristane coincident with tumor inoculation induced a significant increase in MOPC-315 outgrowth. Discussion here is confined to exclusion of nonspecific stress via the hypothalamic-adrenal axis or general immunological paralysis as a sufficient explanation for pristane-enhanced transplantability.

Injection of pristane s.c. constitutes a stress sufficient to produce a severe local inflammatory response. There is ample evidence that stress may enhance or inhibit transplantability of a variety of tumors (17, 21–23). The only documented explanation of such effects involves the hypothalamic-adrenal axis; one expects thymic involution, adrenal hypertrophy, changes in blood leukocyte counts, and duplication of the enhanced transplantability by administration of corticoid or ACTH (13, 24). We have looked for and failed to find such effects in mice given simultaneous injections of pristane and MOPC-315 tumor cells. Possibly, stress may influence tumor outgrowth without the evidence of adrenal excitation, but the effect described here was not reproduced by the stress of injected mineral oil (same side or opposite to the site of PCT inoculation), silica gel or s.c. implantation of cotton. Several authors reported that treatment with complete Freund’s adjuvant, Bacillus Calmette-Guérin, or other adjuvants induced increased tumor growth in viral, chemical, and spontaneous neoplasms (1, 5, 7, 14, 29). However, complete Freund’s adjuvant given in the same regimen as pristane to MOPC-315-challenged mice had no stimulatory effect on tumor outgrowth (data not shown).

Although we have tested only a few transplantable tumors, our data suggest that pristane-enhanced transplantability is not nonspecific; the effect was seen with both plasma tumor cell lines tested (MOPC-315 and MPC-11) but not with the other tumors transplantable to BALB/c mice [DCII chondrosarcoma and P1798 lymphosarcoma (corticoid-sensitive and -resistant)].

The temporal response to pristane is not consistent with some nonspecific stress from the inflammatory response. The response occurs rapidly and lasts much longer than the brief period required during which the host is simultaneously exposed to PCT and pristane. Even a 3-day gap in either direction is sufficient to remove the response.

Paralysis of immunological defense by pristane against tumor growth could also explain increased transplantability. There is ample literature concerned with immunosuppression in pristane-induced plasmacytomas (4, 8–11, 15, 16, 25, 30, 31). However, there are only a few reports regarding the effects of pristane itself on the immune response (12, 20). We looked for the effect of this hydrocarbon on immunological defense as an explanation for the enhanced transplantability, and we found that pristane had little effect on anti-SRBC antibody production. In contrast, pristane administration had altered the splenocyte response to PHA mitogens. A significant decrease in $[^3]H$ thymidine incorporation into PHA-stimulated splenocytes collected from tumor-bearing animals inoculated with PCT and pristane plus PCT or from mice given pristane only was observed. Splenocytes from pristane and pristane plus PCT-inoculated mice decreased the stimulation of normal splenocytes by PHA.

The mechanism involved in pristane-enhanced transplantability of plasmacytomas is still unclear. Attempts are presently being made to clarify the cause of pristane effect.

REFERENCES


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