Regulation of Tumor-induced Myelopoiesis and the Associated Immune Suppressor Cells in Mice Bearing Metastatic Lewis Lung Carcinoma by Prostaglandin E2

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ABSTRACT

The in vitro and in vivo effects of prostaglandin E2 (PGE2) and of its stable analogue, 16,16-dimethyl-PGE2 (dmPGE2), on myelopoiesis and on the myelopoiesis-associated immune suppressor cell activity of mice bearing metastatic Lewis lung carcinoma (LLC-C3) tumors are assessed. In vitro studies showed a reduced susceptibility of bone marrow myeloid progenitor cells (CFC) from LLC-C3 tumor bearers versus normal mice to the growth-inhibitory effects of PGE2. When added to cocultures of bone marrow cells with LLC-C3 supernatants, PGE2 lessened the frequency of CFC and slightly reduced the generation of bone marrow immune suppressor cells. In vivo studies showed that 4 daily injections of dmPGE2 into LLC-C3 tumor-bearing mice caused some reduction in femoral bone marrow CFC and had an insignificant effect on bone marrow suppressor cell activity. In contrast to the relative insensitivity of bone marrow cells of tumor bearers to the effects of PGE2, in vitro studies showed that CFC formation by spleen cells of tumor bearers was readily inhibited by PGE2. Likewise, in vivo studies showed that spleen cells of dmPGE2-treated LLC-C3-bearing mice had a reduction in cellularity, CFC, and the level of spontaneous proliferation; a reduction in suppressor cell activity; and an increase in blastogenesis. Thus, short-term dmPGE2 treatment of LLC-C3-bearing mice limited the tumor-induced splenic myelopoiesis and reduced the associated splenic immune suppressor cell activity.

INTRODUCTION

The progressive growth of tumors in patients and experimental animals results in an aberration in the regulation of both myelopoiesis and immunity. For example, growth in mice of a variety of tumor types, including TS/A mammary adenocarcinoma, LLC1, or 4T0.1 plasmacytoma, resulted in splenomegaly and an increase in myeloid progenitor cells (CFC) (1-10). This tumor stimulation of myelopoiesis was associated with tumor cell production of CSFs. Tumor stimulation of myelopoiesis in mice bearing BMT-11 fibrosarcomas enhanced the ability of injected B16 melanoma tumor cells to colonize and to metastasize (11).

Tumor growth in a host frequently results in reduced immune competence and an increased appearance of immune suppressive cells (12-17). Increased suppressor T-lymphocyte activity was apparent in lymph nodes adjoining melanotic tissue and in the spleens of mice bearing a variety of tumor types (18-20). Suppressive activity of monocytes and macrophages became more prominent during progressive growth of X5563 or MOPC-315 plasmacytomas, methylcholanthrene-induced fibrosarcomas, and of LLC tumors (17, 21-24).

In our studies, we have shown that the decline in immune competence of mice with progressively growing metastatic LLC-C3 tumor cells was associated with the appearance of a sequence of immune suppressor cell populations (5, 16, 17, 24, 25). These suppressor cells were inhibitory to the activities of T-lymphocytes and natural killer cells, both of which are known to be important components of the host anti-tumor defense system. First the immune suppression was mediated by PGE2-producing adherent splenic and peritoneal macrophages (16, 17, 24). However, as tumors became large (≥3 g), the suppressive activity of macrophage-derived macrophages declined as did their production of PGE2. The decline of the PGE2-mediated immune suppressor mechanism coincided with an increased stimulation of myelopoiesis and the appearance of a population of immature bone marrow-derived immune suppressor cells (5, 25). The myelopoiesis stimulation resulted in splenomegaly and in increased numbers of progenitor cells in both the bone marrow and spleen. The myelopoiesis-associated suppressor cells resembled immature cells of the monocyte lineage, and their presence was associated with an increased frequency of monocytic cells in the spleen and peripheral blood (5).

The regulation of myelopoiesis is normally controlled by positive mediators such as the M-CSF, GM-CSF, G-CSF, and interleukin 3 (26-28), and negative regulators such as acidic isoferritins, interferons, and prostaglandins (29-34). Administration of PGE2 to mice or the addition of macrophage-derived PGE to in vitro bone marrow cultures resulted in a reduction in myeloid CFC (35, 36). The PGE2 can also modulate expression of la antigen on stem and myeloid progenitor cells and, in turn, regulate the sensitivity of these cells to inhibition by acidic isoferritins (37-39).

In the current study, a known negative regulator of myelopoiesis, PGE2, was used to minimize the myelopoiesis stimulation which was prominent in mice bearing LLC-C3 tumors. By doing so, we were able to minimize the myelopoiesis-associated immune suppressor activity of spleen cells and, consequently, augment the T-lymphocyte immune competence of the LLC-C3 tumor bearers.

MATERIALS AND METHODS

Medium. The medium used was RPMI-1640 containing 100 units/ml of penicillin, 100 μg/ml of 4(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer solution, 5 × 10⁻³ M 2-mercaptoethanol, 2 mM L-glutamine, and 10% endotoxin-free FBS (Hyclone Laboratories, Logan, UT).

Mice. Six- to 8-wk-old male C57BL/6 mice were used for all studies. The mice were obtained from Cumberland View Farms (Clinton, TN) and then housed at the Hines V. A. animal research facility.

LLC-C3 Tumor Cells (40). Cloned metastatic variant cells of the Lewis lung carcinoma, LLC-C3, were used in all studies. These cells do not produce immune suppressive factors and produce only minimal levels of PGE2. However, the LLC-C3 cells secreted CSF activities which stimulate proliferation of monocytic and monocytic-granulocytic bone marrow CFC (5). In the present studies, the LLC-C3 tumor cells were implanted into mice by dorsal s.c. injection of 5 × 10⁵ cells.

Culture of Bone Marrow Cells with LLC-C3 Supernatant. Normal
bone marrow cells were suspended to 5 x 10^6/ml in medium only or medium containing 20% supernatant from 24-h cultures of 1 x 10^6/ml of LLC-C3 cells. In some of these bone marrow cultures, PGE2 was added to yield 1 ng/ml or 0.1 ng/ml. After 3 days of culture, both the adherent and nonadherent cells were collected and used in CFC and suppressor cell assays.

Treatment of Mice with dmPGE2. At 3 wk after LLC-C3 tumor implantation, when tumors were approximately 20 mm in diameter, normal and tumor-bearing mice were treated daily for 4 days by i.p. injection of diluent or 10 µg of dmPGE2 (Upjohn Diagnostics, Kalamazoo, MI). On the day following the last dmPGE2 treatment, mice were sacrificed, and their spleen cells and femoral bone marrow cells were used. Studies were conducted with at least 5 mice/group.

CFC Assay (41). Bone marrow cells (7.5 x 10^6) or spleen cells (7.5 x 10^6) from individual mice were plated into each 35 x 10-mm tissue culture dish in 1 ml of semisolid supplemented RPMI-1640 medium containing 20% FBS and 0.3% agar (Becto Agar; Difco Laboratories, Detroit, MI). PWMSCS was used as a source of CSF (42) at a final concentration of 10%. This preparation of murine CSF, which was free of known inhibitory molecules, stimulated colonies containing granulocytes (approximately 10%), macrophages (approximately 30%), and granulocytes plus macrophages (approximately 50%). In some assays, various concentrations (100 µg/ml to 1 µg/ml) of PGE2 were added to the dishes. The colonies (>50 cells) were counted after 6 days of culture.

In Vitro Effect of PGE2 Formation by Bone Marrow and Spleen Cells of LLC-C3 Tumor-Bearing Mice. The frequency of CFC in the bone marrow and spleen cells of mice bearing large metastatic LLC-C3 tumors was increased to 1.6-fold and 5.5-fold, respectively, that of normals (Table 1). This increase in the frequency of CFC contributed to an increase in the number of CFC per femur or per spleen. For example, the number of CFC per femur of tumor bearers was increased to 40.2 ± 1.9 x 10^3 as compared to 15.6 ± 2.0 x 10^3 for normals. The number of CFC per spleen of tumor bearers was increased to 25.3 ± 4.4 x 10^3 as compared to 2.2 ± 1.5 x 10^3 for normals. The addition of PGE2 to the cultures of bone marrow or spleen cells resulted in a dose-dependent reduction in the CFC formation. This was evident for cells obtained from either normal mice or LLC-C3 bearers. There was, however, a reduced susceptibility of the bone marrow cells from tumor bearers versus normals to the inhibitory effects of PGE2. A 50% inhibition of CFC formation by bone marrow cells of normal mice was achieved with less than 1 ng/ml of PGE2, while the 50% inhibitory PGE2 dose was approximately 460 ng/ml for bone marrow cells of LLC-C3-bearing mice. In contrast to the relative insensitivity of bone marrow cells of tumor bearers to the inhibitory effects of PGE2, CFC formation by spleen cells of tumor bearers was more readily inhibited by PGE2 with the 50% inhibitory PGE2 dose being approximately 22 ng/ml.

In Vitro Effects of PGE2 on the Frequency of Progenitor Cells and on the Generation of Suppressor Activity in Cultures of Bone Marrow Cells with LLC-C3 Supernatant. Our previous studies showed that LLC-C3 tumor cells secrete CSFs and that the LLC-C3 supernatants induced the appearance of suppressor cells from normal bone marrow cells (5). Culturing bone marrow cells for 3 days in medium containing 20% LLC-C3 supernatant resulted in the persistence of bone marrow progenitor cells which subsequently were able to grow into colonies in the soft agar CFC assay (Table 2), and in the generation of immune suppressor cells which were inhibitory to T-lymphocyte activation (Table 3). The immune suppressor cells which were induced by culture with LLC-C3 supernatants mediate their suppressive activities through a prostaglandin-independent mechanism which was not influenced by the addition of the prostaglandin synthesis inhibitor, indomethacin. For example, in one study the tumor supernatant-cultured bone marrow cells inhibited normal spleen cell blastogenesis to Con-A by 82% in the absence of indomethacin and by 78% in the presence of indomethacin. In contrast to the effects of culturing bone marrow cells with tumor supernatant, culturing bone marrow cells with only medium resulted in a loss of progenitor cells and in marginal levels of suppressor cell activity. The addition of PGE2 to bone marrow cells cultured with either LLC-C3 supernatant or with

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Added PGE2 (ng/ml)</th>
<th>Control mice</th>
<th>LLC-C3 bearers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CFC %-0 PGE2</td>
<td>P</td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>81 ± 9</td>
<td>(100)</td>
<td>131 ± 15</td>
</tr>
<tr>
<td>0.1</td>
<td>62 ± 14</td>
<td>76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1</td>
<td>33 ± 2</td>
<td>41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10</td>
<td>22 ± 7</td>
<td>28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>100</td>
<td>12 ± 7</td>
<td>15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1000</td>
<td>11 ± 7</td>
<td>14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>17 ± 1</td>
<td>(100)</td>
<td>93 ± 11</td>
</tr>
<tr>
<td>0.1</td>
<td>15 ± 4</td>
<td>87</td>
<td>NS</td>
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<tr>
<td>1</td>
<td>13 ± 6</td>
<td>77</td>
<td>NS</td>
</tr>
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<td>10</td>
<td>16 ± 6</td>
<td>92</td>
<td>NS</td>
</tr>
<tr>
<td>100</td>
<td>15 ± 2</td>
<td>88</td>
<td>NS</td>
</tr>
<tr>
<td>1000</td>
<td>7 ± 1</td>
<td>42</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* %-0 PGE2, % of the number of colonies which formed in the absence of added PGE2; NS, not significant.

* Mean ± SD.
Table 2  *In vitro reduction by PGE₂ of the number of progenitor cells in cultures of bone marrow cells with LLC-C3 supernatant*

Normal bone marrow cells were cultured for 3 days with medium or with 20% LLC-C3 supernatant in medium with either no added PGE₂ or with 0.1 ng/ml or 1.0 ng/ml of PGE₂. These cells were then collected and used in a CFC assay by adding 7.5 × 10⁴ cells to 35-mm dishes with 10% (v/v) PWMSCS as a source of CSF.

<table>
<thead>
<tr>
<th>Cells in CFC assay</th>
<th>CFC/dish</th>
<th>PGE₂ effect, (%) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh BM¹</td>
<td>83.7 ± 8.4²</td>
<td></td>
</tr>
<tr>
<td>BM precultured with Medium only</td>
<td>18.3 ± 1.5</td>
<td>-27</td>
</tr>
<tr>
<td>+ 0.1 ng/ml of PGE₂</td>
<td>13.3 ± 0.6</td>
<td>-27</td>
</tr>
<tr>
<td>+ 1.0 ng/ml of PGE₂</td>
<td>9.0 ± 2.6</td>
<td>-51</td>
</tr>
<tr>
<td>BM precultured with LLC-C3 supernatant</td>
<td>59.0 ± 5.2</td>
<td>-25</td>
</tr>
<tr>
<td>+ 0.1 ng/ml of PGE₂</td>
<td>44.3 ± 4.2</td>
<td>-25</td>
</tr>
<tr>
<td>+ 1.0 ng/ml of PGE₂</td>
<td>30.3 ± 5.7</td>
<td>-49</td>
</tr>
</tbody>
</table>

* Values for the number of CFC formed by PGE₂-precultured cells which are significantly different (P < 0.05) from the CFC values for cells precultured in the absence of added PGE₂ are expressed as the percentage of change due to PGE₂.

The addition of PGE₂ to these bone marrow cell cultures also caused a slight reduction in the generation of suppressor cell activity by the LLC-C3 supernatant and in the less prominent suppressor cell activity which was present after culture with only medium (Table 3).

Table 3  *In vitro inhibition of the generation of bone marrow suppressor cells by culture with PGE₂*

Normal bone marrow cells were cultured for 3 days with medium or with 20% LLC-C3 supernatant in medium with either no added PGE₂ or with 0.1 ng/ml or 1.0 ng/ml of PGE₂. The ability of these cells to suppress normal spleen cell blastogenesis to Con-A was measured (Table 5). Treatment of normal mice with PGE₂ resulted in a 56% decrease in CFC per spleen which was due to both a decrease in the frequency of CFC and in the number of nucleated cells in the spleen. Treatment of normal mice with dmPGE₂ also decreased the number of cells per spleen but caused a slight increase in the frequency of splenic CFC. Consequently, dmPGE₂ treatment of normal mice had no effect on the number of CFC per spleen.

In a separate study, the effect of dmPGE₂ treatment on the spleen cell incorporation of [³H]thymidine during the 18 h following removal from tumor-bearing or normal mice was measured and was shown to agree with the above results of the CFC assay. In the absence of any stimulants, the [³H]thymidine incorporated (cpm) by spleen cells of placebo-treated normal and LLC-C3-bearing mice was 13,189 ± 2,551 and 120,100 ± 5,482, respectively. The [³H]thymidine incorporated by spleen cells of dmPGE₂-treated normal and LLC-C3-bearing mice was reduced, respectively, to 9,571 ± 2,719 (not significantly different) and to 81,457 ± 11,622 (P < 0.01).

Effect of Treating Normal and LLC-C3-bearing Mice with dmPGE₂ on Their Spleen Cell Blastogenic Response. The effect of treating normal and tumor-bearing mice with dmPGE₂ on their spleen cell blastogenic response to various doses of Con-A was measured (Table 5). Treatment of normal mice with dmPGE₂ had no effect on blastogenesis in response to 4 μg/ml of Con-A and inhibited blastogenesis in response to 2 or 1 μg/ml of Con-A by 24% and 26%, respectively (P < 0.05). Placebo-treated LLC-C3 bearers were minimally able to respond to Con-A with their blastogenesis being reduced by over 60% as compared to that of normal mice. In contrast to the immune suppressive effect of dmPGE₂ treatment in normal mice, treatment of tumor-bearing mice with dmPGE₂ resulted in a significant restoration of their Con-A responsiveness. For example, dmPGE₂ treatment of tumor bearers restored their responsiveness to 4 μg/ml of Con-A from 37% to 75% of the response by normals.
were also determined and the numbers of CFC per organ were then calculated.

In Experiments 3 to 5, the numbers of nucleated cells in the femoral bone marrow or in the spleen of individual mice was assessed. Each treatment group contained 8 mice.* Ratio of responder normal spleen cells to added suppressor cells.

Effect of dmPGE2 Treatment of Normal and LLC-C3-bearing Mice on the Suppressor Activity of Their Bone Marrow and Spleen Cells. Studies were conducted to determine if dmPGE2 treatment of normal and LLC-C3-bearing mice would minimize the levels of immune suppressor activity of their bone marrow and spleen cells (Table 6). While bone marrow cells of normal mice had some suppressor cell activity, causing 32% suppression of normal spleen cell blastogenesis, bone marrow cells of LLC-C3-bearing mice had more suppressor activity and suppressed blastogenesis by 50%. Treatment of normal mice with dmPGE2 caused a complete elimination of suppressor activity of their bone marrow cells. In contrast, dmPGE2 treatment of tumor-bearing mice tended to only minimally lessen their bone marrow suppressor activity (not statistically significant).

The effects of dmPGE2 administration to tumor bearers on the suppressor activity of their spleen cells differed from the results described above for bone marrow cells. No suppressive activity was present in spleen cells prepared from either placebo-treated or dmPGE2-treated normal mice. In contrast, spleen cells of LLC-C3-bearing mice suppressed normal spleen cell blastogenic response by approximately 76%.

Effect of dmPGE2 Treatment of Normal and LLC-C3-bearing Mice on the Suppressor Activity of Their Bone Marrow and Spleen Cells. Studies were conducted to determine if dmPGE2 treatment of normal and LLC-C3-bearing mice would minimize the levels of immune suppressor activity of their bone marrow and spleen cells (Table 6). While bone marrow cells of normal mice had some suppressor cell activity, causing 32% suppression of normal spleen cell blastogenesis, bone marrow cells of LLC-C3-bearing mice had more suppressor activity and suppressed blastogenesis by 50%. Treatment of normal mice with dmPGE2 caused a complete elimination of suppressor activity of their bone marrow cells. In contrast, dmPGE2 treatment of tumor-bearing mice tended to only minimally lessen their bone marrow suppressor activity (not statistically significant).

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Table 4: Effect of dmPGE2 on the number of CFC in spleen and bone marrow of normal and LLC-C3-bearing mice following bone marrow and spleen cell transplantation. Each treatment group contained 8 mice.* Ratio of responder normal spleen cells to added suppressor cells.

<table>
<thead>
<tr>
<th>Cells</th>
<th>dmPGE2 treatment</th>
<th>CFC/dish (%) of norm</th>
<th>Cells/organ, × 10^7</th>
<th>CFC/organ, × 10^7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>Normals</td>
<td>-</td>
<td>85.3 ± 3.0^a</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>LLC-C3 bears</td>
<td>+</td>
<td>63.4 ± 2.4^a (74)^f</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>Normals</td>
<td>-</td>
<td>13.8 ± 1.3</td>
<td>11.2 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>LLC-C3 bears</td>
<td>+</td>
<td>17.5 ± 1.7^a (127)</td>
<td>7.6 ± 1.3^a (67)</td>
</tr>
</tbody>
</table>

Table 5: Effect of dmPGE2 treatment of LLC-C3 tumor bearers on their Con-A blastogenic response.

<table>
<thead>
<tr>
<th>Cells Source</th>
<th>Con-A (µg/ml)</th>
<th>Placebo</th>
<th>Con-A response of mice treated with dmPGE2</th>
<th>% of change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>Normals</td>
<td>183,160 ± 6,424</td>
<td>177,387 ± 10,811</td>
<td>-3</td>
<td>NS^e</td>
</tr>
<tr>
<td>Splen</td>
<td>Normals</td>
<td>36,386 ± 12,906</td>
<td>136,730 ± 5,096</td>
<td>75</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Splen</td>
<td>LLC-C3 bearers</td>
<td>227,330 ± 36,471</td>
<td>173,037 ± 10,315</td>
<td>24</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 6: Effect of dmPGE2 treatment of LLC-C3 tumor bearers on their suppressor cell activity.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Source</th>
<th>Ratio^b</th>
<th>Medium (µg/mL)</th>
<th>Con-A blastogenic response by spleen cells^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>N/A^d</td>
<td>N/A</td>
<td>148,613 ± 19,501^e</td>
<td>N/A</td>
</tr>
<tr>
<td>BM</td>
<td>Normals</td>
<td>1:1</td>
<td>100,721 ± 10,441</td>
<td>155,456 ± 13,213</td>
</tr>
<tr>
<td>Splen</td>
<td>Normals</td>
<td>1:1</td>
<td>146,626 ± 9,249</td>
<td>137,376 ± 3,046</td>
</tr>
</tbody>
</table>

^a Mean ± SEM.
^b Values for dmPGE2-treated mice are significantly different (P < 0.05) from the value for placebo-treated mice.
^c Numbers in parentheses, values which are significantly different (P < 0.05) from those for normal placebo-treated mice expressed as the percentage of the normal value.
^d The Con-A blastogenic response of individual control and LLC-C3 tumor-bearing mice which had been treated with placebo or with dmPGE2 was measured. Each treatment group contained 8 mice.
^e The percentage of change in Con-A blastogenesis due to dmPGE2 treatment of mice.
^f Level of significance in the difference between the response of dmPGE2-treated mice as compared to the response of placebo-treated mice.
^g Mean ± SEM (cpm).
^h NS, not significant.
^i Numbers in parentheses, values which are significantly different (P < 0.05) from those for normal placebo-treated mice expressed as the percentage of the normal value.
^j The effect of bone marrow and spleen cells from placebo- or dmPGE2-treated normal or LLC-C3-bearing mice on the Con-A responses of normal spleen cells was assessed. Each treatment group contained 8 mice.

^ Ratio of responder normal spleen cells to added suppressor cells.
^c N/A, not available; BM, bone marrow.
^d Mean ± SEM of 3 experiments.
^e Significant (P < 0.05) levels of suppressor cell activity are expressed as the percentage of suppression of the normal spleen cell response by the added cells.
^f For value of dmPGE2-treated mice is significantly (P < 0.05) different from the value for placebo-treated mice.
were less sensitive to the myelosuppressive effects of PGE2 than control mice or to compare the levels of responsiveness of tumor stimulation of myelopoiesis and the associated appearance of immune suppressor cells. As PGE2 is a potent negative regulator of myelopoiesis (31–39), it was used in vitro and in vivo in the presently described studies to restrict the appearance of tumor-induced myelopoiesis and the associated bone marrow-derived suppressor cells.

The results of in vitro studies showed that PGE2 could be effective at inhibiting the tumor-induced myelopoiesis in order to inhibit the appearance of the myelopoiesis-associated immune suppressor cells. The doses at which these effects could be observed, 1 ng/ml and 100 pg/ml, are physiologically pertinent, as the plasma PGE2 levels of normal mice and of mice bearing large tumors are typically between 530 and 600 pg/ml (Ref. 17 and data not shown). These in vitro studies supported the use of PGE2 in LLC-C3 tumor bearers at a time when tumor stimulation of myelopoiesis and the associated appearance of bone marrow-derived suppressor cells were prominent. In the in vivo studies, treatment of tumor bearers with dmPGE2 was restricted to 4 daily injections to (a) minimize any direct macrophage effects on myelopoiesis and suppressor cells. Short-term treatment of tumor bearers with dmPGE2 resulted in some reduction in femoral CFC and had an insignificant effect on the presence of immune suppressor bone marrow cells. However, the dmPGE2 treatment of tumor bearers caused a marked reduction in splenic myelopoiesis and a reduction in splenic immune suppressor cells. The capacity of the dmPGE2 treatments in diminish splenic myelopoiesis and suppressor cell activity prompts further studies to determine whether these effects would be expressed as a measurable reduction in tumor growth. These in vivo results were consistent with the results of in vitro studies showing that bone marrow cells of tumor bearers were less sensitive to the myelosuppressive effects of PGE2 than were either bone marrow cells from normal mice or spleen cells from tumor-bearing mice. It would be of interest to quantitate PGE2 receptors on progenitor cells of tumor bearers versus control mice or to compare the levels of responsiveness of tumor-bearer progenitor cells to PGE2 by assessing either their intracellular levels of cyclic cAMP or the modulation of their expression of la-associated antigen.

The conclusion of our present studies is that short-term treatment of tumor bearers with PGE2 is efficacious in the spleen in limiting tumor-induced myelopoiesis stimulation and the presence of myelopoiesis-associated immune suppressor cells.

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REFERENCES

21. Fuji, T., Igarashi, T., and Kishimoto, S. Significance of suppressor macro-


Regulation of Tumor-induced Myelopoiesis and the Associated Immune Suppressor Cells in Mice Bearing Metastatic Lewis Lung Carcinoma by Prostaglandin E\textsubscript{2}

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