Prostaglandins in Cells of the Lymphoid System in AKR Leukemia

Rashida A. Karmali, T. Wustrow, H. Tzvi Thaler, and R. A. Good

Prostaglandin biosynthesis was therefore studied in 24-hr cultures in vitro at 37°C of peritoneal macrophages, splenocytes, thymocytes, bone marrow, and lymph node cells. AKR mice of 2, 6, and 8 to 12 months of age were studied. Prostaglandin E₁, prostaglandin E₂, 6-keto-prostaglandin F₁₀, and thromboxane B₂ were measured. In cultures of peritoneal macrophages and cells from spleen, thymus, and lymph nodes, the biosynthesis of all five prostaglandin moieties was higher in those cultures prepared from 8- to 12-month-old spontaneously leukemic mice in comparison with those from 2-month-old nonleukemic AKR mice. However, when leukemia was transplanted in 3-month-old AKR mice, synthesis of all five compounds was reduced significantly in cultures of peritoneal macrophages and splenocytes prepared from these 3-month-old leukemic mice. The present data demonstrate abnormalities in prostaglandin synthesis by various cells of the immune system in leukemic mice. However, the nature of these changes was different in cultures of cells from spontaneously leukemic mice from those with transplanted leukemia. Age-related increases in prostaglandin synthesis by various lymphoid cells from spontaneously leukemic AKR mice (8 to 12 months old) occurred at a much earlier age than in BALB/c mice and may be related to the leukemic condition.

INTRODUCTION

AKR mice develop spontaneous lymphoid leukemia relatively late (8 to 12 months) in life, although persistent production of endogenous MuLV occurs throughout life. This finding suggests that age-related changes in the host as well as the relation of the host to endogenous virus may be important in the development of leukemia (3, 11).

The endogenous viruses of AKR mice have been separated into 2 broad groups: ecotropic MuLV (6) and xenotropic MuLV. The expression of these 2 classes of endogenous virus is controlled by mechanisms which are age related (6, 9, 12, 15). At 5 to 6 months of age, AKR mice of the high-ecotropic virus undergo marked increases in the level of expression of MuLV antigens in thymocytes (9). The progressive decline of immune function and increased incidence of cancer, autoimmunity, and infection in aging humans suggest that the development of age-associated diseases may be controlled by immunological factors. Analysis of age-related cellular regulating mechanisms therefore may contribute to our understanding of the pathogenesis of neoplasia that peaks late in life.

Prostaglandins are ubiquitous substances that play an important role in regulating many cellular functions. PGE has been found to be increased in a variety of animal and human tumors (7) and to influence the immunological status of the affected host (5). These observations raise questions about prostaglandin metabolism in AKR leukemia. The present experiments were undertaken to determine whether prostaglandin production was altered in lymphoid cell cultures prepared from leukemic AKR mice. In our studies of BALB/c mice, ranging in age from 3 to 42 months, prostaglandin biosynthesis by most cells of the immune system was increased in mice from around 18 months of age. Much the same has been reported of quantitative measurements of PGE₂ and 6-keto-PGF₁₀ (breakdown product of prostacyclin) (1). We assumed to begin with that this amplification in arachidonic acid metabolism was an age-related phenomenon observed in mice older than 12 months of age. Any increases in prostaglandin biosynthesis by cells of the lymphoid system in 8- to 12-month-old AKR mice would probably be a function of the leukemic condition. We have measured biosynthesis of 5 prostaglandins and related compounds in cultures of lymphoid cells prepared from 2-month-old (no evidence of leukemia), 6-month-old (developing leukemia), and 8- to 12-month-old (macroscopic evidence of leukemia) AKR mice. Two control groups were also included: 3-month-old AKR mice given injections of leukemic or nonleukemic thymocytes. The cells of the lymphoreticular system studied include peritoneal macrophages and mononuclear leukocytes from the spleen, thymus, bone marrow, and lymph nodes.

MATERIALS AND METHODS

Animals. Four-week-old AKR/J mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. These mice were maintained on laboratory chow and used in groups of 6 at 2, 6 and 8 to 12 months of age. Mice used for transplantation of leukemic thymocytes were from the colony at the Sloan-Kettering Institute. Three-month-old AKR mice (n = 6) were given injections of 10⁸ leukemic thymocytes by the i.p. route. After 7 to 10 days, these animals showed symptoms of leukemia (enlarged thymuses, spleens, and lymph nodes) and were used for preparation of cultures of peritoneal macrophages, spleen mononuclear cells, and thymocytes. An additional 6 mice (3 months old) were given injections of 10⁷ nonleukemic thymocytes and were used for cultures of peritoneal macrophages.

Cell Preparation and Culture. The animals were sacrificed by cervical dislocation. Macrophages were isolated by peritoneal lavage with 2 ml of phosphate-buffered saline. Bone marrow cell suspensions were obtained by flushing the tibia, femur, and humerus with cold Roswell Park Memorial Institute Tissue Culture Medium 1640 (Grand Island Biological Co., Grand Island, N.Y.). Single-cell suspensions were also prepared...
from spleen, thymus, and cervical and inguinal lymph nodes. Mononuclear leukocytes were isolated from bone marrow, spleen, thymus, and lymph node suspensions by density gradient centrifugation on Ficoll-Hypaque (Lymphoprep; Nyegaard, Oslo, Norway) by the method of Boyum (2). The viability of the cells was tested by the trypan blue exclusion test.

Quadruplicate cultures of the various lymphoid cells were incubated in v-bottomed microtiter plates at 37° in 5% CO₂ and Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 2% bovine serum albumin at the following concentrations: peritoneal macrophages, 1 × 10⁶ cells; spleen, 2 × 10⁶ cells; lymph node cells, 1.25 × 10⁶ cells; thymocytes, 1.25 × 10⁶ cells; and bone marrow, 2 × 10⁵ cells. In order to facilitate handling of multiple cultures, experiments involving 3-month-old healthy or leukemic AKR mice were restricted to peritoneal macrophages, spleen cells, and thymocytes.

Prostaglandins are synthesized within the cell but are not stored. Instead, they are extruded rapidly into the extracellular space (14). Therefore, at the end of 24 hr, the cultures were centrifuged (50 × g for 7 min) at room temperature, and supernatants from quadruplicate cultures for each organ were pooled for analysis. These supernatants were kept frozen at −70° for measurements of prostaglandins.

**RESULTS**

Peritoneal Macrophages. Synthesis and release of most of the 5 compounds measured, PGE₁, PGE₂, PGF₂α, 6-keto-PGF₁α (metabolite of prostacyclin), and TXB₂ (metabolite of thromboxane A₂) were measured by specific radioimmunoassay. These compounds were selected because they were either found to be major arachidonate products synthesized by macrophages (9) or were related to immunosuppression observed in aging (1, 5, 16). Although PGD₂ is one of the major prostanooids produced in cultures of bone marrow (10), quantitation of this compound was not possible since supplies of PGD₂ antisera were not available for the radioimmunoassay. The procedures for extracting the prostaglandins and details of immunoassay methodology were described earlier (8). Antisera to PGE₁ and PGE₂ were obtained from the Pasteur Institute, Paris, France. Antisera to PGF₂α, 6-keto-PGF₁α, and TXB₂ were raised and characterized in our laboratory. The cross-reaction of PGE₁ and PGE₂ antisera with the respective PGE₂ and PGE₁ standards was approximately 10%; standards to PGF₂α, 6-keto-PGF₁α, and TXB₂ had less than 3% cross-reaction when tested against PGA₁, PGA₂, PGB₁, PGE₁, PGE₂, GF₁α, and 13,14-dihydro-15-keto-PGF₂α. Prostaglandin standards were kindly supplied by Dr. John Pike, The Upjohn Co., Kalamazoo, Mich.

The sensitivity of the assays has been found to be approximately 10 pg/ml.

**Statistical Methods.** Assays were done on mice from each age group in 11 replicate experiments. Analysis of variance was applied to the log-prostaglandin levels, testing the age main effect against the residual variance (age-by-experiment interaction). Prostaglandin measurements in mice with transplanted leukemia were done for 3 lymphoid cell types only. Student’s t test was applied to prostaglandin levels.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Peritoneal macrophages</th>
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<tbody>
<tr>
<td>Control</td>
<td>10⁷ nonleukemic thymocytes (pg/ml culture medium)</td>
</tr>
<tr>
<td>PGE₁</td>
<td>3,221 ± 577.8³</td>
</tr>
<tr>
<td>PGE₂</td>
<td>32,006 ± 3,659.9</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>542 ± 75.8</td>
</tr>
<tr>
<td>6-keto-PGF₁α</td>
<td>26,067 ± 3,928.1</td>
</tr>
<tr>
<td>TXB₂</td>
<td>1,676 ± 147.8</td>
</tr>
</tbody>
</table>

* p = 0.003; PGE₁, p < 0.001; PGE₂, p = 0.004; PGF₂α, p = 0.018; and TXB₂, p = 0.017 (Chart 1). All animals of the 8- to 12-month age group were macroscopically leukemic with enlarged thymuses, spleens, and lymph nodes. On the other hand, there was no evidence of leukemia in any of the 2-month-old AKR mice. In order to clarify the significance of this increase in the 5 compounds in the pathogenesis of leukemia, similar cultures were studied from 3-month-old AKR mice with transplanted leukemia. Synthesis and release of all 5 compounds were decreased significantly in these cultures compared with cultures prepared from the 3-month-old healthy AKR mice (PGE₁, p = 0.003; PGE₂, p < 0.001; PGF₂α, p = 0.02; 6-keto-PGF₁α, p < 0.001; and TXB₂, p = 0.056) (Table 1). However, in the group receiving injections of nonleukemic thymocytes, there was no evidence of leukemia, and levels of all 5 compounds were comparable to the control macrophage cultures. These results suggest that the increased biosynthesis of 5 prostaglandin moieties in macrophage cultures from spontaneously leukemic 8- to 12-month-old AKR mice is probably a function of age.

**Spleen Mononuclear Leukocytes.** Cultures prepared from leukemic mice (8 to 12 months old) release significantly higher levels of PGE₁ (p < 0.038), PGE₂ (p < 0.044), PGF₂α (p < 0.002), and TXB₂ (p < 0.002) in comparison with healthy 2-month-old AKR mice (Chart 2). Additionally, it was found that spleen cultures prepared from 6-month-old AKR mice produced even higher amounts of PGE₁, PGE₂, and PGF₂α than older mice (8 to 12 months). The increased levels of PGE₁, PGE₂, and PGF₂α may...
Prostaglandins and AKR Leukemia

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Age (months)

Chart 2. Leukocytes (2 × 10^6) in Roswell Park Memorial Institute Tissue Culture Medium 1640 supplemented with 2% bovine serum albumin were incubated in microtiter plates at 37°C in 5% CO₂. The cultures were centrifuged after 24 hr and analyzed for prostaglandin content. Cultures prepared from leukemic mice (8 to 12 months old) released significantly higher levels of PGE (p < 0.008), PGE₂ (p < 0.004), PGF₂α (p < 0.002), and TXB₂ (p < 0.002) in comparison with those from apparently healthy 2-month-old AKR mice (Student's t test). O, PGE₁; □, PGE₂; +, PGF₂α; ×, 6-keto-PGF₁α; ○, TXB₂.

Table 2
Spleen mononuclear cell cultures

Statistical analysis was by Student's t test, n = 6.

<table>
<thead>
<tr>
<th></th>
<th>Control (pg/ml culture medium)</th>
<th>10⁷ leukemic thymocytes (pg/ml culture medium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₁</td>
<td>3,897 ± 477.7</td>
<td>117 ± 97.6</td>
</tr>
<tr>
<td>PGE₂</td>
<td>32,408 ± 3,491.0</td>
<td>5,157 ± 829.7</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>1,566 ± 171.7</td>
<td>104 ± 26.6</td>
</tr>
<tr>
<td>6-keto-PGF₁α</td>
<td>4,415 ± 682.2</td>
<td>1,234 ± 274.5</td>
</tr>
<tr>
<td>TXB₂</td>
<td>3,748 ± 307.4</td>
<td>1,023 ± 284.6</td>
</tr>
</tbody>
</table>

*Mean ± S.E.

p < 0.001.

p < 0.01.

be related to the pathological process manifested in the AKR mice beginning at about 6 months of age. However, an age-related component is more likely to be associated with the increase of these compounds, since all 5 compounds were decreased in cultures prepared from 3-month-old AKR mice in which leukemia was induced by transplantation of leukemic thymocytes (PGE₁, p < 0.001; PGE₂, p < 0.001; PGF₂α, p < 0.001; 6-keto-PGF₁α, p < 0.001; and TXB₂, p < 0.001) (Table 2).

Thymocytes. Cultures from leukemic mice (8 to 12 months old) released significantly higher concentrations of PGE₁ (p < 0.009), PGE₂ (p < 0.002), PGF₂α (p < 0.001), 6-keto-PGF₁α (p < 0.001), and TXB₂ (p < 0.004) in comparison with cultures prepared from healthy 2-month-old AKR mice (Chart 3). Similar cultures from 3-month-old AKR leukemic mice released higher amounts of PGE₁, PGF₂α, and 6-keto-PGF₁α (p < 0.01) in comparison with cultures prepared from healthy 3-month-old AKR mice (Table 3). Since development of T-cell leukemia in AKR mice involves the thymus in particular, the present findings indicate that changes in prostaglandin biosynthesis in cultures of thymic cells are related, at least in part, to leukemia.

Lymph Node Cells. In comparison with cultures from healthy 2-month-old mice, cultures prepared from leukemic mice (8 to 12 months old) released significantly higher levels of some prostaglandins: PGE₁ (p < 0.044) and PGF₂α (p < 0.006) (Chart 4).

Bone Marrow Mononuclear Cells. There was no significant difference in prostaglandin release by cultures prepared from 2-month- and 8- to 12-month-old AKR mice (Chart 5). However, levels of PGE₁, PGE₂, 6-keto-PGF₁α, and TXB₂ were increased in cultures from 6-month-old mice. It therefore seems improbable that this increase in synthesis of some prostaglandins is age related. The significance of the changes observed remains unclear but suggests that prostaglandins may play an important part in the pathogenesis of leukemia which is developing.

DISCUSSION

In vitro production of 5 prostanooids was measured in cells from 5 different sites at 3 different age groups in AKR mice. The cells cultured from each organ were lymphoid cells. In addition, adherent peritoneal macrophages were cultured for analysis.
Excessive prostaglandin production, particularly of the E series, has been reported in several types of malignant tissues in both humans and experimental animals (for review, see Ref. 7). Because all normal tissues are capable of synthesizing prostaglandins, it is logical to expect that cancerous cells should do so. In the present study, prostaglandin production was increased significantly in cultures of peritoneal macrophages, spleen mononuclear cells, thymocytes, and lymph nodes prepared from spontaneously leukemic AKR mice (8 to 12 months old). However, we and others have observed increases with age in prostaglandin release by normal lymphoid cells in culture (1). It was not certain that the changes in prostaglandin synthesis observed in cultures from 8- to 12-month AKR mice were related to the leukemic condition. Therefore, similar cultures were prepared from 3-month-old AKR mice with transplanted leukemia.

Following transplantation of $10^7$ leukemic thymocytes in 3-month-old AKR mice, the leukemic disease was apparent within 10 days. Cultures of peritoneal macrophages, spleen mononuclear cells, and thymocytes were prepared from these and control mice before the leukemic animals succumbed to the disease by about 14 days. Biosynthesis of all 5 prostaglandin moieties measured was decreased significantly in cultures of peritoneal macrophages and spleen mononuclear cells prepared from leukemic mice in comparison with those prepared from control mice. However, in macrophage cultures from 3-month-old AKR mice given injections of $10^7$ nonleukemic thymocytes, levels of the 5 prostaglandins were similar to those in the control group. These observations suggest that the mechanisms controlling prostaglandin synthesis and release in cultures of lymphoid cells from spontaneously leukemic AKR mice are different from those from AKR mice with transplanted leukemia. In the latter case, the changes are most probably related to the leukemic condition, since cultures from AKR mice given injections of nonleukemic thymocytes were similar to the control group.

The data presented also indicate that the increased levels of prostaglandins in cultures from 8- to 12-month-old AKR mice with spontaneous leukemia may be related to age. Our studies of BALB/c mice ranging in age from 3 to 42 months showed that increased prostaglandin biosynthesis by most cells of the immune system was apparent only in mice around 18 months of age and older. Much the same has been reported of quantitative measurements of PGE2 and 6-keto-PGF1α. In the AKR strain of mice, the age-related increase in prostaglandin release seems to occur at a much earlier age than in the BALB/c mice. The possibility that these changes in prostaglandin synthesis may be linked to the pathogenesis of leukemia cannot be ruled out.

Development of T-cell leukemia in AKR mice involves the thymus in particular. Therefore, the increased biosynthesis of 5 of 5 compounds in thymocyte cultures from 8- to 12-month-old leukemic mice (spontaneous) and 3 of 5 compounds in thymocyte cultures from 3-month-old leukemic mice (transplanted) demonstrates clearly that changes in prostaglandins are related, at least in part, to the leukemic condition. To our knowledge, this is the first demonstration of thymic changes in prostaglandin synthesis in leukemic AKR mice. Whether it is the tumor cells, the host reaction to the tumor, or a change in lymphoreticular function that is associated with these changes in ability of the lymphoreticular cells to produce prostaglandins may be quite important in considering the pathogenetic mechanisms of certain cancers. This is especially so, since certain prostaglandins (E series in particular) seem to be able to influence immunological function significantly (4, 5). Furthermore, analysis of prostaglandin sensitivity of granulocyte-macrophage-committed colony-forming cells from patients with chronic myeloid leukemia has indicated that an abnormal growth regulation response to inhibition by PGE
may play a role in leukemogenesis (13). The findings from the present studies urge further penetrating analysis of the association of prostaglandin production by malignant cells and of changes in prostaglandin production as a reaction to developing lymphoreticular and epithelial cancers.

ACKNOWLEDGMENTS

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REFERENCES

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