Factors Influencing Augmentation and/or Acceleration of Lymphoreticular Tumors in Mice by Benzo(a)pyrene Treatment

S. D. Vesselinovitch, A. P. Kyriazis, N. Mihailovich, and K. V. N. Rao

SUMMARY

The response of lymphoreticular tissues to a single i.p. injection of benzo(a)pyrene was studied in the first generation of C57BL/6J x C3HeB/FeJ F1 and C3HeB/FeJ x A/J F1 mice. Groups of 1-, 15-, and 42-day-old animals of both sexes received 75 or 150 μg of the carcinogen per g body weight.

After a period of approximately 90 weeks, a high incidence (up to 43%) of reticulum cell sarcomas was observed in C57BL/6J x C3HeB/FeJ F1 mice treated with benzo(a)pyrene at 42 days of age. Animals treated with carcinogen at younger ages had a lower incidence of reticulum cell sarcomas. These sarcomas showed marked cellular pleomorphism and were classified into histiocytic, epitheloid-nodular, reticulocytic, and fibrocytic forms according to the predominant cell type. Lymphomas of thymic and extrathymic lymphoid origin and leukemia of granulocytic type were seen in a descending order of frequency.

Control animals of either strain that were killed at 90 weeks of age were basically free of lymphoreticular tumors, while those kept under observation up to 170 weeks developed these tumors in 24% (C57BL x C3H F1) and 10% (C3H x A/J F1), respectively. Studies revealed that the augmentation and/or acceleration of development of lymphoreticular neoplasms and specifically reticulum cell sarcomas by benzo(a)pyrene was dependent upon the strain and sex of mice used and the age at which the animals were exposed to carcinogen.

INTRODUCTION

This study deals with the development of lymphoreticular tumors in mice exposed to B(a)P. As an integral part of a series of studies on carcinogenesis by various carcinogens in neonatal, infant, and young adult mice (7, 14, 15), B(a)P was used in this investigation as representative of polycyclic carcinogenic hydrocarbons because of its widespread occurrence in our environment and its potential causal relationship to cancer in man. Its tumorigenic effects were assessed following single i.p. injections, in contrast to other studies in which the animals were fed B(a)P for protracted periods of time (8, 9).

In the course of the present study, a spectrum of tumors of lymphoreticular tissues developed. The most frequently observed tumor type was reticulum cell sarcoma, which was followed by lymphomas of extrathymic and thymic origin. Presented data relate to histological appearance, primary site of origin, incidence, and metastatic behavior of lymphoreticular tumors in 2 hybrid strains of mice exposed to B(a)P at birth, infancy, or young adulthood.

MATERIALS AND METHODS

The 1st generation of C57BL/6J x C3HeB/FeJ F1 (hereafter called B6C3F1) and C3HeB/FeJ x A/J F1 (hereafter called C3A/JF1) mice was used. The parent strains had been purchased from The Jackson Laboratory, Bar Harbor, Maine, and the appropriate crosses were bred in our laboratory. After weaning at 4 weeks of age, the animals were separated by sex, housed in plastic cages in groups of 10, and kept in temperature-controlled quarters. Sanicel was used as bedding material, and Rockland mouse diet and water were given ad libitum. Mice were weighed at 2-week intervals and inspected throughout the period of the experiment for the presence of any external neoplasm and of other symptoms indicating the development of internal tumors and/or nonspecific pathological changes affecting their health.

B(a)P was purchased from Aldrich Chemical Co., Milwaukee, Wis., and its purity was tested by absorption spectrophotometry. Carcinogen was dissolved in trioctanoin and was injected i.p. into groups of 1-, 15-, and 42-day-old mice of both sexes. Each animal received a single i.p. injection of 75 or 150 μg (delivered in a volume of 0.01 ml) of B(a)P per g body weight. A total of 611 males and 550 females received the above-described treatment. No acute toxic effects were observed following administration of carcinogen. Due to development of tumors, animals lived on the average 89 weeks. Control groups that received 0.01 ml of either 0.9% NaCl solution or trioctanoin showed good survival. One set of controls was killed at 90 weeks of age, while the other was left to live their life-span (170 weeks).
Complete autopsies were performed on all animals, and grossly visible lesions were recorded. Autopsy specimens were fixed in a 10% buffered formalin solution, embedded in paraffin, cut in 5-μm sections, and stained with hematoxylin and eosin.

RESULTS

The pathological changes of the lymphoreticular tissues of both B(a)P-treated and control animals were studied microscopically. Specific morphological cellular changes, obliteration of nodular architecture, infiltration of the capsule and surrounding tissues, and distant metastases to other organs were considered as standard criteria of cancer. A few cases in which microscopic changes were focal and limited to areas adjacent to inflammatory process were not included in the present neoplastic tabulation, since it was difficult to rule out their nonspecific reactive nature.

Classification. In this study, tumors of the lymphoreticular tissues were classified into 3 groups: (a) lymphomas, (b) leukemias, and (c) reticulum cell sarcomas. The lymphomas included lymphocytic (Fig. 1), lymphoblastic, and stem-cell lymphomas, and those of thymic origin (Fig. 2). However, since the distinction between lymphomas of either lymphocytic or lymphoblastic type and the corresponding leukemias was not possible in most cases, all lesions considered to be leukemias of lymphoid origin were classified with the lymphomas. Consequently, only leukemias of myelocytic origin were represented in the leukemic group.

Whereas the above tumors showed remarkable cellular uniformity and consistency, the reticulum cell sarcomas resulting from B(a)P treatment were characterized by marked cellular pleomorphism. Therefore, this class of tumors was subdivided according to the predominant cell type into (a) histiocytic, (b) epithelioid-nodular, (c) reticulocytic, and (d) fibrocytic form.

Histiocytic tumors showed a predominance of histiocytic elements (Fig. 3), with occasional presence of multinucleated giant cells (Fig. 4). Epithelioid tumors consisted of cellular elements resembling epithelioid cells. This variant appeared in general in nodular pattern (Fig. 5), although more diffuse infiltrations were occasionally observed. Reticulocytic form showed both reticulocytic and lymphocytic cellular components. Most of these tumors, however, had predominant reticulocytic elements (Fig. 6). Fibrocytic tumors showed the presence of fibrocytic elements and the production of collagen by the supporting stroma (Fig. 7); multinucleated giant cells were occasionally present, alternating with fibrocytic elements and primitive reticular cells (Fig. 8).

In classifying a lesion as a reticulum cell sarcoma, the following criteria were used. The reticulum cell sarcoma was the only neoplasm presented and no other tumor was detected in any one of the examined tissues, the giant cells of the histiocytic type were intermingled with primitive reticular cells and neoplastic histiocytes, and the tumor showed areas of transition to pure histiocytic or epithelioid type.

Tumor Response. The control series of animals that were killed at 90 weeks of age showed only 2% of the lymphoreticular tumors, regardless of the strain and sex (Table 1). However, in the series left to live its life-span (up to 170 weeks), the incidence of spontaneous lymphoreticular tumors increased to 24% in B6C3F1, and to 10% in the C3A/JF1 hybrids (15). However, this increase in the incidence of spontaneous lymphoreticular tumors with the animals' age was due in 67% of the cases to the development of lymphocytic lymphomas.

In both mouse strains there was no statistically significant difference in tumor incidence between groups of the same age that received 70 or 150 μg of B(a)P per g body weight. Therefore, both "dose" groups for each strain were combined, and the incidence of lymphoreticular tumors was presented in relation to age and sex in Tables 2 and 3 for B6C3F1, and C3A/JF1, strains, respectively. As indicated in the last columns, B(a)P-treated animals lived, on the average, 89 weeks.

The overall incidence of lymphoreticular tumors in carcinogen-treated B6C3F1 hybrids was 32% (104 of 326) and 53% (148 of 281) for males and females, respectively (p < 0.001). This sex-associated difference was primarily due to differential development of reticulum cell sarcomas, which was also the predominant histological entity. Thus, regardless of age of animals at treatment, 79 of 326 males (24%) and 103 of 281 females (37%) developed reticulum cell sarcoma (p < 0.001).

In addition to sex, the response of the mice to the administration of B(a)P was associated with the age at which the animals were exposed to the carcinogen (Table 2). Thus, when the treatment was initiated within the 1st 24 hr after birth, 23% of males and 43% of females developed lymphoreticular tumors. The incidence rose to 29% for males and 54% for females in the group of animals treated at 15 days of age. When B(a)P was given at 6 weeks of age, however, 45% of males and 61% of females developed lymphoreticular tumors. This positive age-associated difference in the incidence of tumors between the 1- and 42-day-old mice was statistically significant in both sexes (p < 0.001 for males; p < 0.05 for females).

Of all lymphoreticular tumors, reticulum cell sarcoma accounted for 76% (79 of 104) cases in males and 70% (103 of 148) in females. Detailed histological study showed that, out of the 182 reticulum cell sarcomas, the primary sites of their origin were liver in 118 cases (65%), spleen in 42 cases (23%), lymph nodes in 16 cases (9%), and gastrointestinal tract in 3 cases (2%). In the group of females, 3 cases originated in the uterus. Of the reticulum cell sarcomas, 156 (86%) metastasized extensively to various organs, whereas 26 (14%) were localized and showed no evidence of metastasis. Table 4 lists the distribution and frequency of metastasis of the 4 types of reticulum cell sarcomas to various organs.

Lymphomas of thymic origin (25 cases) were more aggressive, as indicated by their widespread metastases. They metastasized primarily to the spleen, liver, lymph nodes, lungs, and kidneys with equally high frequency, followed by metastases to the adrenals, salivary glands, retroperitoneal soft tissues, and brain. Of the 37 lymphomas of nonthymic origin, 31 (84%) were of the lymphocytic and 6 (17%) were of the lymphoblastic or stem-cell type. Their
Development of Lymphoreticular Tumors in B(a)P-treated Mice

Table 1

Incidence of lymphoreticular tumors in B6C3F1 and C3A/JF1 control mice

Due to the very small number of lymphoreticular tumors, all control animals were combined regardless of sex or whether they were treated with 0.9% NaCl solution or trioctanoin. All animals were sacrificed at 90 weeks of age.

<table>
<thead>
<tr>
<th>Type of tumor</th>
<th>Incidence no.*</th>
<th>No. metastasizing</th>
<th>Liver</th>
<th>Spleen</th>
<th>Lymph nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonthymic lymphoma (lymphocytic)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Reticulum cell sarcoma</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Histiocytic</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Epithelioid</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reticulocytic</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Fibrocytic</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Absolute number; there were a total of 500 mice between both hybrids.

Table 2

Incidence (percentage) of lymphoreticular tumors in B6C3F1 mice following administration of B(a)P at 1, 15, or 42 days of age

<table>
<thead>
<tr>
<th>Age at treatment (days)</th>
<th>No. of animals</th>
<th>% reticulum cell sarcoma</th>
<th>% thymic lymphoma</th>
<th>% nonthymic lymphoma</th>
<th>% granulocytic leukemia</th>
<th>% total lymphoreticular tumors</th>
<th>Age at death (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>112</td>
<td>15.2</td>
<td>3.6</td>
<td>3.6</td>
<td>0.9</td>
<td>23.2</td>
<td>85 ± 2</td>
</tr>
<tr>
<td>15</td>
<td>112</td>
<td>22.3</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>28.6</td>
<td>85 ± 2</td>
</tr>
<tr>
<td>42</td>
<td>102</td>
<td>36.3</td>
<td>5.9</td>
<td>5.9</td>
<td>0.0</td>
<td>45.1</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>89</td>
<td>23.6</td>
<td>5.6</td>
<td>13.5</td>
<td>0.0</td>
<td>42.7</td>
<td>117 ± 3</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>42.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>54.0</td>
<td>92 ± 2</td>
</tr>
<tr>
<td>42</td>
<td>92</td>
<td>43.5</td>
<td>5.4</td>
<td>12.0</td>
<td>0.0</td>
<td>60.9</td>
<td>97 ± 3</td>
</tr>
</tbody>
</table>

* B(a)P dissolved in trioctanoin was injected i.p. once at specified ages; each animal received 75 or 150 µg of carcinogen per g of body weight.
* Mean ± S.E.

Table 3

Incidence (percentage) of lymphoreticular tumors in C3A/JF1 mice following administration of B(a)P at 1, 15, or 42 days of age

<table>
<thead>
<tr>
<th>Age at treatment (days)</th>
<th>No. of animals</th>
<th>% reticulum cell sarcoma</th>
<th>% thymic lymphoma</th>
<th>% nonthymic lymphoma</th>
<th>% granulocytic leukemia</th>
<th>% total lymphoreticular tumors</th>
<th>Age at death (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>114</td>
<td>3.5</td>
<td>1.8</td>
<td>0.9</td>
<td>0.0</td>
<td>6.1</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>15</td>
<td>109</td>
<td>8.3</td>
<td>3.7</td>
<td>1.8</td>
<td>0.0</td>
<td>13.8</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>42</td>
<td>62</td>
<td>4.8</td>
<td>0.0</td>
<td>1.6</td>
<td>0.0</td>
<td>6.5</td>
<td>87 ± 2</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>101</td>
<td>3.0</td>
<td>1.0</td>
<td>2.0</td>
<td>0.0</td>
<td>7.9</td>
<td>78 ± 2</td>
</tr>
<tr>
<td>15</td>
<td>105</td>
<td>13.3</td>
<td>5.7</td>
<td>1.0</td>
<td>0.0</td>
<td>21.0</td>
<td>89 ± 2</td>
</tr>
<tr>
<td>42</td>
<td>72</td>
<td>20.8</td>
<td>1.4</td>
<td>0.0</td>
<td>5.5</td>
<td>27.8</td>
<td>89 ± 2</td>
</tr>
</tbody>
</table>

* B(a)P dissolved in trioctanoin was injected i.p. once at specified ages; each animal received 75 or 150 µg of carcinogen per g of body weight.
* Mean ± S.E.

The primary sites of origin were spleen (19 cases, 51%), gastrointestinal tract (9 cases, 24%), lymph nodes (7 cases, 19%), and liver (2 cases, 5%).

In the C3A/JF1 strain, the pattern and distribution of lymphoreticular tumors were similar to those observed in the B6C3F1 mice. The overall incidence of these tumors, however, differed markedly. Thus, it was found that only 9% of males and 18% of females developed lymphoreticular tumors. This strain difference was primarily due to the lower development of reticulum cell sarcomas in C3A/JF1 mice (48 of 563, 9%) than in B6C3F1 hybrids (182 of 607, 27%; p < 0.001). An increase in the overall incidence of
lymphoreticular tumors with the age of the animals was observed only in C3A/JF1 females (Table 3). This strain also showed a lower frequency of metastasizing reticulum cell sarcomas, which might have been partly due to the relatively higher incidence of the nodular epithelioid-cell type in this series (Table 4).

**DISCUSSION**

Due to the marked histological variations of reticulum cell sarcomas that emerged following carcinogenic treatment, it was impossible to classify them in only 2 types (A and B) according to earlier reports (1, 2). Consequently, the reticulum cell sarcomas were subclassified into 4 subgroups, regardless of their histogenesis. However, the introduced terms were used in a descriptive histological sense denoting the predominant cell type and not the purity of the lesion. The fibrocytic form of reticulum cell sarcoma was described herein as a new entity, being characterized by fibrocytic elements and the production of collagen by the supporting stroma. The production of collagen was regarded as a reactive, reparative process which apparently could be related to the relatively localized nature of this neoplastic form. Consequently, the used subclassification of reticulum cell sarcoma possesses both morphological and biological significance. The latter characteristic becomes especially obvious from data presented in Table 4, which show that the histiocytic type was biologically the most aggressive and the fibrocytic form the least aggressive, as indicated by their differential metastatic rates.

From the reported data, several phenomena regarding lymphoreticular tissue carcinogenesis became apparent. Thus, 2 strains differed in their capability of developing lymphoreticular tumors, either spontaneously or following administration of B(a)P. The B6C3F1 hybrids proved to be significantly more responsive to B(a)P treatment than the C3A/JF1 mice. This finding is in agreement with previous observations that showed that mouse strains vary considerably in their susceptibility to the induction of lymphoreticular tumors which occasionally would parallel their spontaneous tendencies (12). In the present study, B(a)P potentiated and accelerated the development of reticulum cell sarcomas that occurred with low frequency in the aged, nontreated, controls.

In addition to the strain, the sex of the animals influenced development of lymphoreticular tumors due to the administration of B(a)P. Thus the incidence of lymphoreticular tumors was higher in the B6C3F1 females than in males. This sex difference was observed consistently within each age group and could not be attributed exclusively to the longer life-span of the female animals. Thus, animals treated with B(a)P at 6 weeks of age (Table 2) had the same longevity, regardless of sex, and yet females developed significantly more lymphoreticular tumors than males ($p < 0.05$).

The response of the lymphoreticular tissues to the administration of B(a)P was also found to be related to the age of animals at treatment. Thus animals receiving B(a)P at 6 weeks of age were significantly more responsive than those exposed to carcinogen within 24 hr of birth or at 15 days of age. This age-associated pattern has also been observed in the same mouse strains following administration of other chemical carcinogens (15). However, the observed age-associated trend was not due to the enhanced emergence of thymic lymphomas but, rather, to increased development of reticulum cell sarcomas. This morphological pattern is in contrast to the effect of irradiation on the development of lymphoreticular tumors, which was predominantly associated in most mouse strains with the development of lymphomas of thymic origin, the infant animals being more susceptible than the adults (3). In addition, irradiation-induced lymphomas usually showed a positive dose-dependent latent period (10), which has not been detected in the present study following B(a)P treatment. These variations, however, may indicate a difference in the mode of action or mechanism between ionizing irradiation and B(a)P-induced leukemogenesis (4–6, 11, 13).

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**Table 4**

**Frequency, distribution and metastatic behavior of the 4 types of reticulum cell sarcomas in B(a)P-treated mice**

This table does not include 3 reticulum cell sarcomas that originated in the uterus and 3 in the gastrointestinal tract. All of these were of the histiocytic type.

<table>
<thead>
<tr>
<th>cell type</th>
<th>B6C3F1 Frequency</th>
<th>Metastasizing</th>
<th>C3A/JF1 Frequency</th>
<th>Metastasizing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Histiocytic</td>
<td>111</td>
<td>61</td>
<td>106</td>
<td>95</td>
</tr>
<tr>
<td>Epithelioid</td>
<td>28</td>
<td>15</td>
<td>17</td>
<td>61</td>
</tr>
<tr>
<td>Reticulocytic</td>
<td>31</td>
<td>18</td>
<td>28</td>
<td>90</td>
</tr>
<tr>
<td>Fibrocytic</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>176</td>
<td>100</td>
<td>153</td>
<td>86</td>
</tr>
</tbody>
</table>

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In regard to the present study, it became obvious that B(a)P treatment augmented and/or accelerated development of reticulum cell sarcoma, depending upon the strain and sex of mice and the age at which the animals received the carcinogen. Actual mechanism(s) of such effects, however, should be the objective of future studies. However, because most reticulum cell sarcomas originated in liver (Table 4), it is intriguing to speculate that both the susceptibility of target tissue and the enzymatic competence necessary for optimal concentration of ultimate carcino-genic entity coincided with the most responsive conditions for development of this tumor type.

REFERENCES


Fig. 1. Malignant lymphoma, lymphocytic type, infiltrating lung parenchyma. The tumor cells are slightly larger than mature lymphocytes. The nuclei are darkly stained, prominent nucleoli are seen in some of the cells, and cytoplasm is practically nonexistent. This tumor originated in the mesenteric lymph node and spread to the liver, kidney, and lungs. Lung parenchyma is extensively replaced by neoplastic cells. Lung. H & E, × 400.

Fig. 2. Malignant lymphoma, thymic origin. Large immature lymphocytes characterized by uniformity in size and shape. The nuclei are round and well stained with prominent nucleoli. Atypical mitotic figures are a frequent finding with this type of lymphoma. H & E, × 400.

Fig. 3. Reticulum cell sarcoma, histiocytic type. Note diffuse growth of neoplastic histiocytes which possess abundant cytoplasm and show nuclear polymorphism. Spleen. H & E, × 400.

Fig. 4. Reticulum cell sarcoma, histiocytic type with multinucleated giant cells. Spleen. H & E, × 200.

Fig. 5. Reticulum cell sarcoma, nodular-epithelioid type. Liver. H & E, × 300.

Fig. 6. Reticulum cell sarcomas, reticulocytic type. Neoplastic reticulum cells have well-defined nuclear membranes, show broad cellular polymorphism, and abnormal clumping of nuclear chromatin. Lymph node. H & E, × 500.

Fig. 7. Reticulum cell sarcoma, fibrocytic type. The tumor cells closely packed, arranged in longitudinal bundles, swirling formations, and assuming in certain areas conspicuous nodular pattern. Liver. H & E, × 200.

Fig. 8. Reticulum cell sarcoma, fibrocytic type, with multinucleated giant cells present. Aggregation of giant cells and elongated fibroblast-like cells is obvious. The tumor cells are immature, and the reticulum cells are, as well, rather primitive (arrows). Lymph node. H & E, × 325.
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