Leukemoid Reaction in BALB/c Mice Bearing Primary Tumors Induced by 3-Methylcholanthrene

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ABSTRACT

During the growth of five of ten primary tumors that were induced by 3-methylcholanthrene in BALB/cMk and BALB/cMk × C57BL/6 F1 mice, leukemoid reactions, characterized by increase in number of granulocytes in peripheral blood, and splenomegaly were observed. However, no such reactions occurred in five C57BL/6 mice bearing primary tumors induced by 3-methylcholanthrene. The results of leukemoid reactions in mice bearing 3-methylcholanthrene-induced primary tumors are compared with the reactions found in mice bearing transplanted tumors.

INTRODUCTION

Leukemoid reactions that appeared during the growth of certain tumors transplanted into mice have been described (1, 2, 4, 7–10, 12). However, there were no reports on the reaction in mice bearing primary tumors. We previously reported on the general findings of leukemoid reactions in BALB/cMk mice bearing 10 different lines of transplantable fibrosarcomas. The reaction was also induced in BALB/cMk × C57BL/6 F1 mice (hereafter called CB6F1) mice by transplantation of these tumors (7, 10).

This present paper reports a leukemoid reaction in mice bearing primary MCA2-induced tumors and compares it with the reaction found in mice bearing transplanted tumors.

MATERIALS AND METHODS

Mice. BALB/cMk, C57BL/6, CB6F1 mice were used. Male and female BALB/cMk mice were bred and supplied by the Laboratory for Breeding of Experimental Animals, Hokkaido University, Sapporo, Japan. C57BL/6 mice were supplied by Ohmura Animal Supply Company, Kanagawa, Japan. CB6F1 mice were bred in our laboratory.

Tumors. Primary tumors were induced by s.c. injection of 0.2 ml olive oil containing 1.0 mg MCA in 15 mice 2 months of age (5 BALB/cMk, C57BL/6, and CB6F1). Transplantable tumors were obtained from mice bearing primary tumors just before their death from tumors. Nine tumors in the first passage were transplanted s.c. in 5- to 6-month-old mice by trocar inoculation and they were used for the experiments.

Experimental Methods. Tumor growth in mice was measured with calipers and expressed as a mean tumor diameter, equal to one-half the sum of the minimal and maximal diameters of the tumor. Peripheral blood smears and imprint preparations of bone marrow or spleen were stained with May-Giemsa. When mice bearing tumors showed a mean WBC over 3 x 10⁵/cu mm in peripheral blood accompanied by the appearance of immature cells of the blast stage, we considered this a positive leukemoid reaction. Organs obtained at autopsy, the spleen in particular, were weighed and examined histologically.

As controls, 10 to 20 normal male and female 5- to 6-month-old mice of each strain were used for examinations of peripheral blood and histology.

RESULTS

Leukemoid Reactions in Mice Bearing Primary Tumors. Fibrosarcomas appeared in 15 mice 63 to 111 days after injection of MCA, and mice died 35 to 65 days after the appearance of these tumors. As shown in Table 1, leukemoid reactions were observed in 2 of 5 BALB/cMk mice and in 3 of 5 CB6F1 mice 30 to 38 days after tumor appearance. There was no reaction in any of 5 C57BL/6 mice. The reaction, characterized by granulocytosis and splenomegaly (Fig. 1), was similar to that found in mice bearing transplanted tumors previously reported (7, 10). Bone marrow smears showed intense hypercellularity and an increase in the number of myeloid elements (Fig. 2). Histological examination in mice positive for the reaction revealed that the structure of the spleen was changed, resulting in replacement of normal spleen elements by a proliferation of myeloid cells (Fig. 3). Liver (Fig. 4), lungs, and kidneys were infiltrated with granulocytes. No tumor metastases were observed in any organs and tissues. However, the intensity of reaction was lower than that in mice bearing transplanted tumors. WBC were less than 1 x 10⁵/cu mm, and spleens weighed less than 0.6 g even at the maximal stage of tumor growth. While all the tumors grew to more than 25 mm in diameter, there were no differences of tumor size between mice with positive and negative leukemoid reactions.

Leukemoid Reactions in Mice Bearing Transplanted Tumors. All 13 BALB/cMk mice and 9 CB6F1 mice bearing transplanted tumors showed leukemoid reactions. As shown in Table 1, a characteristic finding obtained from the experiment was the appearance of the reaction in mice bearing transplanted CMT-10 or CBMT-2 tumors that had not induced the reaction in mice bearing these primary tumors. The intensity of reaction in mice bearing transplanted tumors was usually higher than that in mice bearing primary tumors. None of 15 C57BL/6 mice bearing transplanted tumors showed leukemoid reaction.

DISCUSSION

Although there have been reports on leukemoid reactions...
in mice bearing transplanted tumors (1, 2, 4, 7–10, 12), little is known about the reaction in mice bearing primary tumors. Therefore, a question has arisen whether this reaction in mice bearing tumors is valuable as a model for that found in human patients (3, 6). This study showed the occurrence of the leukemoid reaction in BALB/cMk and CB6F1 mice bearing primary tumors induced by MCA. However, their frequencies and intensities of the reaction were lower than that found in mice bearing transplanted tumors. We suspect, although the evidence is far from clear, that some toxic effects by MCA on hematopoietic organs and tissues in the host as described by Stjernswärd (11) might be responsible for the low frequency and intensity of this reaction in mice bearing primary tumors.

Another characteristic finding obtained from this study was the absence of the reaction in C57BL/6 mice bearing MCA-induced primary tumors or transplanted tumors. These results would suggest again that some host factors in BALB/cMk mice are partially responsible for the induction of leukemoid reaction as described previously (7). Furthermore, possible involvement of endogenous leukemogenic viruses, which were activated by MCA, might be responsible for the development of the reaction in BALB/cMk and CB6F1 mice (6). The factors influencing leukemoid reaction in mice bearing primary tumors induced by MCA require further investigation.

**REFERENCES**


### Table 1

<table>
<thead>
<tr>
<th>Strains</th>
<th>Tumors</th>
<th>Maximal WBC (no./cu mm)</th>
<th>Spleen wt (g)</th>
<th>Grade of reaction</th>
<th>Maximal WBC (no./cu mm)</th>
<th>Spleen wt (g)</th>
<th>Grade of reaction</th>
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<tbody>
<tr>
<td>BALB/cMk</td>
<td>CMT-8</td>
<td>49,500</td>
<td>0.31</td>
<td>+</td>
<td>88,000 ± 11,800</td>
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<td>CMT-9</td>
<td>60,500</td>
<td>0.46</td>
<td>++</td>
<td>152,500 ± 15,600</td>
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<td>+++</td>
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<td></td>
<td>CMT-10</td>
<td>28,000</td>
<td>0.22</td>
<td>–</td>
<td>56,000 ± 8,600</td>
<td>0.42</td>
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<td>CMT-11</td>
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<td>CMT-12</td>
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<td>0.25</td>
<td>–</td>
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<tr>
<td></td>
<td>None</td>
<td>15,000 ± 1,100</td>
<td>0.11 ± 0.02</td>
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<td>C57BL/6</td>
<td>BMT-2</td>
<td>15,500</td>
<td>0.21</td>
<td>–</td>
<td>21,500 ± 1,300</td>
<td>0.17</td>
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<td>BMT-3</td>
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<td>0.10</td>
<td>–</td>
<td>26,000 ± 1,800</td>
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<td>BMT-4</td>
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<td>0.20</td>
<td>–</td>
<td>19,500 ± 1,700</td>
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<td>BMT-5</td>
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<td>–</td>
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<td>0.12 ± 0.01</td>
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<td>CB6F1</td>
<td>CBMT-1</td>
<td>38,000</td>
<td>0.36</td>
<td>+</td>
<td>128,500 ± 28,600</td>
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<td>CBMT-2</td>
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<td>73,000 ± 8,400</td>
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<td>CBMT-5</td>
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<td>0.13 ± 0.02</td>
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</table>

**a** Grade of reactions for mean WBC/cu mm: +, 30,000 to 50,000; ++, 50,000 to 100,000; ++++, 100,000 to 500,000.

**b** WBC and spleen weight at the time when tumors were 25 mm in diameter.

**c** Mean ± S.E.

**d** NT, not tested.

**e** Value in 10 to 20 mice of each strain and 5 to 6 months of age.
Leukemoid Reaction in Mice Bearing Primary Tumors

Fig. 1. BALB/cMk mouse bearing MCA-induced primary CMT-9 tumor (arrow) with splenomegaly (double arrows).

Fig. 2. Bone marrow smear from the mouse bearing primary CMT-9 tumor showing marked myelopoiesis. May-Giemsa, ×450.

Fig. 3. Spleen of the mouse bearing primary CMT-9 tumor showing intense myelopoiesis. H & E, ×450.

Fig. 4. Liver of the mouse bearing primary CMT-9 tumor showing some myelopoietic centers. H & E, ×450.
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