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Host Genetic Factors Influencing the Occurrence of Leukemoid Reaction in BALB/cMk Mice Bearing Transplanted Tumors[1]

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ABSTRACT

Host genetic factors influencing the occurrence of leukemoid reaction in BALB/cMk strain mice bearing transplanted tumors were studied. A Mendelian hybridization experiment was performed with BALB/cMk and C57BL/6 strains, and their strain hybrids: the first filial (F_1) generation hybrids; the second filial (F_2) generation hybrids; and the backcrosses to the two parental strains. The results of these studies suggested a genetic regulation of leukemoid reaction occurrence in BALB/cMk mice bearing transplanted tumors. Genes permissive to the occurrence of the reaction seemed to be dominant to their nonpermissive alleles.

INTRODUCTION

We reported previously on the general findings of leukemoid reaction characterized by granulocytosis and splenomegaly in BALB/cMk mice bearing primary and transplanted syngeneic fibrosarcomas (10, 11, 14). The reaction was also induced in BALB/cMk × C57BL/6 F_1 (hereafter called $CB6F_1$) or C57BL/6 × BALB/cMk F_1 (hereafter called $B6CF_1$) mice by primary and transplanted fibrosarcomas (10, 11). These results suggested that some host factors as well as tumor factors were responsible for the induction of leukemoid reaction in tumor-bearing BALB/cMk mice. In this paper further studies of the leukemoid reaction in BALB/cMk mice bearing transplanted tumors are dealt with, in reference to the host genetic factors influencing the occurrence of the reaction, by means of hybridization experiments involving BALB/cMk and C57BL/6 mice.

MATERIALS AND METHODS

Mice. 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 Co., Kanagawa, Japan. BALB/cMk and C57BL/6 mice were mated to produce the reciprocal F_1 hybrid mice. These F_1 generation mice were mated either *inter se*, to produce the F_2 generation mice, or to one or the other of the parental strains to produce reciprocal backcross progeny. All hybrid mice were bred in our laboratory.

Tumors. A transplanted fibrosarcoma, CMT-6, induced by 3-methylcholanthrene in BALB/cMk mice and a transplanted fibrosarcoma, BMT-6, induced by 3-methylcholanthrene in C57BL/6 mice were used for the experiment. Mean survival times of susceptible mice bearing transplanted tumors were 50.1 days with CMT-6 and 46.2 days with BMT-6.

Experimental Methods. Tumor fragments were implanted under the skin of the back with a trocar in 3- to 4-month-old mice. Tumor growth in mice was measured with calipers and expressed as 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 were stained with May-Giemsa. When mice bearing tumors showed a WBC count over 5×10^4/cu mm in a mean value in peripheral blood accompanied by the appearance of immature cells of the blast stage, we considered this a positive leukemoid reaction. Mice were observed for 120 days after transplantation of tumors.

As controls 10 to 20 normal males and females of each strain and of the hybrids, 3 to 4 months old, were used for examination of peripheral blood.

Statistical Analysis. Significance was determined by the χ^2 test.

RESULTS

Leukemoid Reaction Produced in Mice Bearing CMT-6 BALB/c Tumors. Two parent BALB/cMk and C57BL/6 strains and their strain hybrids were given transplants of CMT-6 BALB/c tumors and were tested for tumor growth and leukemoid reaction. As seen in Table 1, while growth-positive and reaction-positive mice were not observed among C57BL/6 mice (0 of 15), all BALB/cMk mice (20 of 20) were growth and reaction positive. In $CB6F_1$ and $B6CF_1$ hybrids, all mice (23 of 23) were growth and reaction positive. Segregants that were growth and reaction negative were found among the F_2 and C57BL/6-backcross mice. The ratio of growth- and reaction-positive mice to growth- and reaction-negative mice approximated a 3:1 ratio (22:8; $p > 0.8$) in the F_2 generation, and a 1:1 ratio (14:12; $p > 0.8$) in the C57BL/6-backcross progeny. Neither growth-positive and reaction-negative mice nor growth-negative and reaction-positive mice were found. We observed no effect of such variables as sex and litter size in the occurrence of leukemoid reaction.

Mice in the parental C57BL/6, F_2 generation, and C57BL/6-backcross groups that were CMT-6 tumor growth and leukemoid reaction negative were again made tumor bearing with BMT-3 C57BL/6 tumors and were tested for tumor growth and leukemoid reaction. As shown in Table 1, growth-positive mice were observed in all C57BL/6 (15 of 15), F_2 generation (12 of 12), and C57BL/6-backcross mice. However, no reaction-positive mice were found.

As previously reported, the leukemoid reaction occur-

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rence, which was recognized by the increase in the peripheral WBC, was related only to the tumor size and not to the period of tumor bearing (11). Tumor growth was similar in parent strains and their strain hybrids. We found no evidence of the so-called hybrid resistance phenomenon (2).

Leukemoid Reaction Produced in Mice Bearing BMT-3 C57BL/6 Tumors. Two parental strains and their strain hybrids into which were transplanted BMT-3 C57BL/6 tumors were tested for tumor growth and leukemoid reaction. As shown in Table 2, while no BALB/cMk mice (0 of 15) were growth positive and reaction positive, all C57BL/6 mice (20 of 20) were growth positive. However, no C57BL/6 mice (0 of 20) were reaction positive. In $CB6F_1$ and $B6CF_1$ hybrids, all mice (21 of 21) were growth and reaction positive. Segregants that were growth and reaction negative, and that were growth positive and reaction negative, were found among the F_2 and BALB/cMk-backcross mice. While the ratio of growth-positive to growth-negative mice approximated a 3:1 ratio (19:7; $p > 0.8$) in the F_2 generation, a 2:1 (12:7; $p > 0.8$) reaction-positive to reaction-negative ratio was obtained. In the F_2 generation 7 growth-positive and reaction-negative mice were found. In the BALB/cMk-backcross progeny, the ratio of growth- and reaction-positive to growth- and reaction-negative mice approximated a 1:1 ratio (14:11; $p > 0.5$). No growth-negative and reaction-positive mice were found.

Mice that were BMT-3 tumor growth negative and leukemoid reaction-negative in the parental BALB/cMk, F_2 generation, and BALB/cMk-backcross mice were again made tumor bearing with CMT-6 BALB/c tumors and were tested for tumor growth and leukemoid reaction. As shown in Table 2, growth- and reaction-positive mice were observed in all BALB/cMk (15 of 15), F_2 generation (7 of 7), and BALB/cMk-backcross (11 of 11) mice. Again we observed no effect of such variables as sex and litter size on the occurrence of the leukemoid reaction.

These results, the segregation of positive or negative occurrence of leukemoid reaction in BALB/cMk mice, were in good agreement with a single dominant gene model ($p > 0.5$) and did not fit a 2-gene interpretation ($p < 0.05$).

DISCUSSION

Leukemoid reaction has been found in humans and animals bearing tumors that do not involve the hematopoietic tissues (1, 4–7, 9–14). Some factors influencing the occurrence of leukemoid reaction have been investigated (3, 10, 11). However, no reports have dealt with the host genetic factors influencing the reaction. As previously reported (10, 11) we found the leukemoid reactions in all BALB/cMk, $CB6F_1$, and $B6CF_1$ mice bearing transplanted

Table 1

Tumor growth and leukemoid reaction occurrence in parental strains of BALB/cMk and C57BL/6 mice and their hybrid progeny into which were transplanted CMT-6 BALB/c tumors, or mice that rejected the CMT-6 tumor and into which were successively transplanted BMT-3 C57BL/6 tumors

	Transplanted tumors											
	CMT-6 BALB/c						BMT-3 C57BL/6[a]					
		Sex			Sex			Sex			Sex	
Mice	No. that died of tumor/no. used	M	F	No. of reaction-positive/no. used[b]	M	F	No. that died of tumor/no. used	M	F	No. of reaction-positive/no. used	M	F
BALB/cMk (C)	20/20	10	10	20/20	10	10						
C57BL/6 (B6)	0/15	0	0	0/15	0	0	15/15	8	7	0/15	0	0
$CB6F_1$ and $B6CF_1$	23/23	10	13	23/23	10	13						
$CB6F_1$ and $B6CF_2$	22/30	10	12	22/30	10	12	8/8	4	4	0/8	0	0
B6-backcross	14/26	6	8	14/26	6	8	12/12	6	6	0/12	0	0

[a] BMT-3 C57BL/6 tumors were transplanted into mice 30 days after rejection of CMT-6 BALB/c tumor.
[b] WBC count was over 5×10^4/cu mm at the time when tumor was 25 mm in diameter.

Table 2

Tumor growth and leukemoid reaction occurrence in parental strains of BALB/cMk and C57BL/6 mice and their hybrid progeny into which were transplanted BMT-3 C57BL/6 tumors, or mice that rejected the BMT-3 tumor and into which were successively transplanted CMT-6 BALB/c tumors

	Transplanted tumors											
	BMT-3 C57BL/6						CMT-6 BALB/cMk[a]					
		Sex			Sex			Sex			Sex	
Mice	No. that died of tumor/no. used	M	F	No. of reaction-positive/no. used[b]	M	F	No. that died of tumor/no. used	M	F	No. of reaction-positive/no. used	M	F
BALB/cMk (C)	0/15	0	0	0/15	0	0	15/15	7	8	15/15	7	8
C57BL/6 (B6)	20/20	10	10	0/20	0	0						
$CB6F_1$ and $B6CF_1$	21/21	9	12	21/21	9	12						
$CB6F_2$ and $B6CF_2$	19/26	11	8	12/26	6	6	7/7	3	4	7/7	3	4
C-backcross	14/25	8	6	14/25	8	6	11/11	5	6	11/11	5	6

[a] CMT-6 BALB/c tumors were transplanted into mice 30 days after rejection of BMT-3 C57BL/6 tumors.
[b] WBC count was over 5×10^4/cu mm at the time when tumor was 25 mm in diameter.

BALB/c fibrosarcomas and in all $CB6F_1$ and $B6CF_1$ mice bearing transplanted C57BL/6 fibrosarcomas. These results suggested that some host factors as well as tumor factors were partially responsible for the induction of the leukemoid reaction. Present genetic studies were aimed at these host factors influencing the reaction occurrence.

The results obtained from these studies suggest that BALB/cMk mice may carry a single dominant gene that confers the occurrence of leukemoid reaction and that the occurrence is not affected by such variables as sex and litter size of these parent and hybrid mice. However, these results were based on experiments with only BALB/cMk and C57BL/6 mice. Additional genetic studies with other mouse strains are required. The results also suggest that the genetic factor that plays a role in the occurrence of the leukemoid reaction in BALB/cMk mice might be related to the major histocompatibility complex of this strain of mice because all mice that died from CMT-6 BALB/c tumor also evidenced the reaction. However, much more extensive genetic studies, including studies with H-2 typing and congenic mice, would be needed to draw this conclusion. Furthermore, possible involvement of endogenous leukemogenic viruses might be responsible for the development of the reaction in BALB/cMk mice because some reports demonstrate that the presence of endogenous leukemogenic viruses and virus-related antigens is subject to host gene regulation (8, 15). More specific investigation of the tumor and host factors influencing the occurrence of leukemoid reaction in BALB/cMk mice will be continued.

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