ANTIBODIES AGAINST METHYLCHOLANTHRENE SARCOMA CELLS IN THE BLOOD OF INTACT MICE

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Cytotoxic and membrane-fluorescence tests in micromodifications were used to detect antibodies against tumor cells in the blood of BALB/c and C57BL mice in autologous and isologous systems. In the blood of intact BALB/c and C57BL mice these tests revealed antibodies against autologous and isologous tumor cells released from sarcomas induced in these mice by methylcholanthrene. In an autologous system the antibody level against tumor cells in BALB/c mice was lower than in an isologous system in the same mice, and also lower than in C57BL mice in an autologous system. The level of these antibodies in autologous and isologous systems was the same in C57BL mice.

Antibody formation is an indication of the antitumor response of an organism. To determine antitumor antibodies in the blood of animals with a neoplasm the hemagglutination test [13], the primary component of complement-fixation test (C^1) [12], globulins labeled with radioactive iodine [10], the colony-formation-inhibition test [6], and the cytotoxic [5, 7] and membrane-fluorescence [2, 9] tests are used.

The last two tests are particularly important, for they can detect antibodies against insoluble surface antigens which determine the antitumor and transplantation reactions of the organism. Admittedly, using the cytotoxic test Möller [9] and Lezhneva [2] were unable to detect antibodies in the blood of mice immunized with syngeneic methylcholanthrene-induced sarcoma tissue, but they found these antibodies in the serum of mice of various strains by means of the membrane-fluorescence test.

Lezhneva [2] observed the presence of antibodies against isologous tumor cells by means of the fluorescence test in a very small percentage of sera of normal CC57W and CC57BR mice.

In an autologous system the present writers detected antitumor antibodies in the blood serum of BALB/c mice during the development of a tumor induced by methylcholanthrene [4, 11]. The appearance of antitumor antibodies in the blood of the mice was recorded as an increase in the cytotoxic (CI) and fluorescence (FI) indices compared with the blood serum of intact mice. A definite level of antibodies against tumor cells was observed in the blood serum of the intact mice.

It was therefore decided to investigate the available evidence on the presence of antibodies in the blood of normal BALB/c and C57BL mice against autologous and isologous tumor cells.

EXPERIMENTAL METHOD

Altogether 46 BALB/c mice and 23 C57BL mice were used. Before injection of the carcinogen, 0.8 ml of blood was taken from the mice from the retro-orbital sinus under open ether anesthesia; the sera prepared from the blood were sealed in ampules, frozen, and kept at −56° until use.

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TABLE 1. Values of CI and FI in Blood Serum of Normal BALB/c and C57BL Mice against Autologous and Isologous Tumor Cells

Line of mice	Number of mice	CI	System	Number of mice	FI
	28	0,25±0,03 (0,20—0,30)	Autologous	13	0,058±0,009 (0,042—0,074)
BALB/c	18	0,35±0,02 (0,32—0,38) $P<0,02$	Isologous	10	0,078±0,009 (0,062—0,094) $P<0,05$
C57BL	12	0,36±0,01 (0,34—0,38) $P<0,001$	Autologous	9	0,074±0,002 (0,070—0,078) $P_1<0,05$
	11	0,37±0,02 (0,33—0,41)	Isologous	7	0,072±0,003 (0,066—0,078)

Note. P) significance of differences between CI and FI in blood serum of BALB/c mice in autologous and isologous systems; P_1) significance of differences between CI and FI for C57BL and BALB/c mice in an autologous system.

The tumor cells were separated by trypsinization and diluted with balanced saline solution (BSS) consisting of 0.14 g $CaCl_2$, 8 g NaCl, 0.4 g KCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.06 g KH_2PO_4, 0.06 g Na_2HPO_4, 1 g glucose, 100,000 units penicillin and 0.1 g streptomycin.

The cytotoxic and membrane-fluorescence tests were carried out in micromodifications as developed in Professor Klein's Laboratory (Karolinska Institute, Stockholm, Sweden). Full details of the method of performing the cytotoxic test were published by the writers previously [11].

Fluorescence Test. To 2×10^5 tumor cells 0.025 ml of the test serum was added, the mixture was shaken and incubated for 30 min at 37°C, twice washed with 1% gelatin solution in BSS, and centrifuged for 1-1.5 min at 5,000 rpm. After the addition of 0.03 ml antimouse fluorescent serum the samples were incubated at 37°C for 20 min, after which the cells were washed twice with 1% gelatin solution in BSS and once in plain BSS. A drop of the cell suspension was dried on a slide, one drop of a 50% solution of glycerol in BSS was added, a coverslip was applied secured with paraffin wax around its edges, and the specimen was examined under the microscope with a 90× objective, using nonfluorescent immersion oil. The number of fluorescent and nonfluorescent cells was counted and the fluorescent index calculated.

EXPERIMENTAL RESULTS

It can be concluded from the results in Table 1 that the blood serum of intact BALB/c and C57BL mice contains antibodies against autologous and isologous tumor cells.

In an autologous system CI ($P < 0.001$) and FI ($P < 0.05$) for BALB/c mice were statistically significantly lower than for C57BL mice. In addition, in BALB/c mice CI ($P < 0.02$) and FI ($P < 0.05$) in an autologous system were statistically significantly lower than in an isologous system.

Antibodies detected with the aid of tumor cells in the blood serum of intact mice could be those antibodies which, in Grabar's opinion [1] perform the function of removing damaged cells from the body. Under the influence of methylcholanthrene it may be that antigens identical to those formed during ordinary cell death are formed on the surface of the cells. These antibodies can also be found with the aid of tumor cells. Further, the antibodies detected could be antibodies against a latent virus, bound with the mouse cells and activated by the carcinogen, or against an antigen induced with this virus. The hypothesis is based on the observations of Klein and Klein [7], who showed that antibodies against Moloney virus are present in 29% of intact BALB/c mice. Finally, the possibility cannot be ruled out that before administration of the carcinogen antibodies can be detected in the blood of mice against isoantigens left on the surface of tumor cells during carcinogenesis.

Differences between the strains of mice for the antibody titer in autologous systems are evidently connected with differences in the ability of the mice of these strains to produce antibodies [3], while the differences between the autologous and isologous systems in BALB/c mice are probably due to the residual heterozygosis within this strain [8].

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