POSITIVE COOMBS TESTS IN MICE AFTER TRANSPLANTATION OF SYNGENEIC TUMORS

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A combination of autoimmune reactions and tumor growth is observed quite frequently in clinical and experimental oncology [9, 11, 13]. The writers showed previously [1] that in some cases autoantibodies, detectable by the indirect Coombs' test, are produced in mice during growth of transplantable tumors, and their peak coincides with the period before the tumor is yet palpable. Clinical data on the presence of positive direct Coombs tests (PCT) in nonhematogenous tumors are scanty [6, 19]. Meanwhile PCT have been observed in many investigations dealing with tumors of hematogenous nature [17], particularly frequently in a precancerous disease currently being actively studied, namely angioimmunoblastic lymphadenopathy [10]. Experimental detection of PCT is also mainly associated with tumors of the hematopoietic system, such as lymphomas in AKR and NZB mice, and so on [7, 14].

The aim of the present investigation was to attempt the screening of mice with various tumors, mainly nonhematogenous, for the presence of a direct PCT.

EXPERIMENTAL METHOD

Mice of the following lines — 129, C57BL/6I, (CBA × C57BL/6I)F_1, and A/Sn, aged 2-6 months — were used. The following syngeneic tumors were used: teratocarcinoma TB-24, at the 14th passage (129); melanoma B16 and leukemia EL-4 (C57BL/6I); hemangiopericytoma (HAPC), at the 13th, 19th, and 20th passages; methylcholanthrene-induced sarcoma S-90, at the 2nd passage (F_1); spontaneous mammary gland carcinoma M-2, 38th passage (A/Sn). The TB-24 and M-2 tumors and mice of the inbred line 129 were generously presented by V. M. Senin's group. Leukemia EL-4 was maintained in the ascites form. The tumor tissue was pressed through a Kapron sieve into Hanks' solution and injected into mice in a dose of 0.2-0.3 ml of a 5-10% suspension. To obtain a single-celled suspension, the suspension of HAPC was additionally filtered through three layers of gauze. The HAPC cells were irradiated on a "Stebel" apparatus (GUPOS), with a ^{137}Cr source, mean dose rate of irradiation in the working volume 630 ± 20 rads/min. The cells were irradiated in a dose of 5000 rads. At different times after inoculation of the tumor, about 0.1 ml of blood was taken from the retro-orbital sinus of the mice into physiological saline (PS) with heparin (50 units/ml). Erythrocytes were washed three times in 15-20 volumes of PS and a 1% suspension was prepared. Erythrocytes of each mouse were tested separately. Rabbit antiserum against mouse immunoglobulins (Ig), obtained from Cederlane (Canada), in dilutions of 1/4 and 1/8 or rabbit antiserum against mouse IgC1, generously provided by E. V. Sidorova (Laboratory of Chemistry and Biosynthesis of Antibodies, N. F. Gamaleya Institute of Experimental Medicine, Academy of Medical Sciences of the USSR), were used as the antiglobulin reagent. Hemagglutination was carried out in our own modification [1]. Erythrocyte suspension in a volume of 10 µl was introduced into wells of a Falcon 3034 plate, 5 µl of antiglobulin serum was added to each well, and the plates were incubated at 37°C for 40-45 min. Erythrocytes of each mouse were tested in duplicate or triplicate. The plate was then kept at room temperature at an angle of about 45° for 10-15 min, after which the reaction was read. An example of the results of one such test is given in Fig. 1. Sometimes, to increase the sensitivity of the method, after incubation of erythrocytes with rabbit antiserum 5 µl of sheep's antiserum against rabbit globulins was added to the wells and incubation was repeated. Sheep antiserum (N. F. Gamaleya Institute of Experimental Microbiology, Batch 2) was used in a dilution of 1/10 in PS to remove nonspecific reactions against mouse antigens or was absorbed with solid residue of erythrocytes of intact mice (in a ratio

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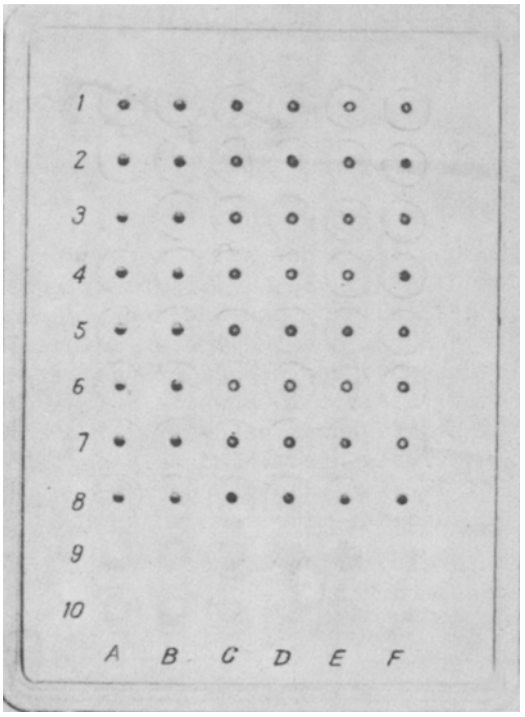

Fig. 1. Example of results of microhemagglutination test. Wells of two vertical rows on the left and bottom wells of the two rows on the right show negative reaction (erythrocytes were shifted toward one edge of the bottom of the well). In the other cases the test was positive (erythrocytes remain uniformly distributed or form a ring at the bottom of the well).

TABLE 1. Induction of Direct PCT in a C57BL/6I—EL-4 System

Expt. No.	Time of taking blood (number of days after transplantation of tumor)	Intact time	Recipients of tumor	P
1	5	1/8	8/8(0/8)	<0,01
	15	1/8	6/8(8/8)	0,02
	32	0/8	4/8(8/8)	0,04
	Total for all times	2/24	18/24	<0,001
2	12	2/10	8/10(6/10)	0,01
	20	2/10	5/10(10/10)	>0,05
	27	2/10	4/10(10/10)	>0,05
	Total for all times	6/30	17/30	<0,01
3	13	1/10	13/20(20/20)	<0,001
4	9	0/10	0/10(0/10)	—
	17	0/10	6/10(10/10)	<0,001
	24	2/9	5/6(6/6)	<0,05
	Total for all times	2/29	11/26	0,002
	Total for four experiments	11/93	59/100	<0,001

Legend. Here and in Tables 2 and 3: numerator indicates number of mice with PCT, denominator — number of mice in group; in parentheses: numerator — number of mice with palpable tumor, denominator — number of mice in group. Tumor inoculated subcutaneously in doses of 10^3 (Expt. No. 1), 10^5 (Expt. No. 3), and 10^2 (Expts. Nos. 2, 4). Recipients were males aged 2-3 months (Expts. Nos. 1, 2, and 4) and females aged 6 months (Expt. No. 3).

TABLE 2. Induction of Direct PCT in a (CBA × C57BL/6I)F_1—HAPC System

Expt. No.	Time of taking blood (number of days after transplantation of tumor)	Intact mice	Recipients of irradiated tumor cells	Recipients of living tumor cells	P
1	4	2/10		12/12(8/12)	0,0001
	11	3/10		11/11(11/11)	0,001
	18	2/10		4/5(5/5)	0,04
	Total for all times	7/30		27/28	<0,001
2	4	2/9	5/9	6/9(1/9)	>0,05*
	15	2/9	6/9	7/6(9/9)	0,03*
	32	0/9	0/9		
	Total for all times	4/27	11/27	13/18	<0,001*

Legend. Mice were inoculated subcutaneously with 750,000 tumor cells each. Tumors at the 13th and 20th passages respectively were used. In Experiment No. 1, females were used, in Experiment No. 2 — males; the age of the mice was 3-4 months. Tumor cells were irradiated in a dose of 5000 rads. Asterisks indicate comparison between groups of intact mice and recipients of living tumor cells. Differences in frequency of PCT between intact control and recipients of irradiated tumor cells were not significant in all cases ($P > 0.05$).

TABLE 3. Detection of PCT on Different Tumor Models

Line of mice, sex, age	Tumor	Time of taking blood (number of days after transplantation of tumor)	Intact mice	Recipients of tumor	P
Female A/Sn, 3 months	M-2/38	20	1/6	5/6(6/6)	0,04
Male C57BL/6I, 3 months	B 16	7	1/8	7/9(4/9)	0,01
		16	1/8	9/9(9/9)	<0,001
		Total for all times	2/16	16/18	<0,001
Male 129, 4 months	TB-24 (14th passage)	18	1/7	7/7(0/7)	0,002
		48	1/7	10/10(10/10)	<0,001
		Total for all times	2/14	17/17	<0,001
Female F_1, 3 months	S-90 (2nd passage)	21	1/8	8/16(16/16)	>0,05
		35/11*	3/8	5/8(0/8)**	4/8(8/8)***
		59/35*	0/8	3/7(1/7)	1/2(2/2)
		101/77*	1/8	0/6(0/6)	
		Total for all times	5/32	13/26	<0,02

Legend. All tumors were inoculated intramuscularly. In experiment with S-90 the tumors were removed from eight experimental mice on the 24th day, and in 6 mice they had grown successfully. One asterisk: in numerator — number of days after transplantation, in denominator — number of days after operation; two asterisks — mice undergoing operation; three asterisks — control of tumor growth.

of 10:1 by volume) at 4°C overnight, and then used in a dilution of 1/4-1/8. Both modifications of the hemagglutination test were verified repeatedly beforehand in a standard system with mouse anti-H-2 antibodies. Intact mice of the same pool were used as the control. The results were subjected to statistical analysis by the χ^2 method and its modification for small samples.

EXPERIMENTAL RESULTS

The results showed (Tables 1-3) that PCT are found significantly more often in mice with tumors than in the intact control population. The phenomenon was observed in six tumor models and four lines of mice. In the experiments with TB-24 and B-16, PCT were found before the appearance of palpable tumors; in the case of TB-24 differences in the frequency of PCT between intact mice and the group of mice with the "precancerous" state were statistically significant. The results of the experiment with TB-24 confirm our previous observations [1] on the appearance of an autoimmune component during the growth of this tumor.

The results can be explained in various ways. We know that tumors maintained for a long time by subculture often have many "passengers" (viruses, and so on), capable of inducing

autoimmune reactions [2, 5] and, in particular, of synthesizing antierythrocytic antibodies. In the present experiments T-24 and HAPC had gone through fewer than 20 passages, and S-90 through only two; without ruling out this explanation, therefore, we consider that it is unlikely or incomplete. During rough mechanical dispersion of the tumor much necrotic material is obtained and, according to some workers [3], it can induce a burst of PCT in the recipients. However, this explanation does not apply to the EL-4 model, when the animals were inoculated subcutaneously with a very dilute suspension of living ascites cells. In addition, experiments with HAPC and other preliminary data are evidence that injection of killed tumor cells does in fact induce PCT, which then gradually disappear, unlike in the group of animals with tumors, in which PCT as a rule are observed regularly until death of the mice. According to abundant clinical and experimental evidence, during tumor growth soluble antigen–antibody complexes (immune complexes – IC) circulate in the bloodstream [8, 16]. The possibility of adsorption of IC on erythrocytes cannot be ruled out on a priori grounds. However, we were unable to find in the literature any assertion of this fact during tumor growth. Yet according to preliminary data, in our experiments the frequency of PCT fell much more slowly after removal of the tumor from the mice, i.e., when IC were gradually disappearing from the bloodstream, than during persistence of IC.

Without ruling out a possible role of all these factors, we suggest the following explanation of the results. It has now been shown on numerous experimental models and, in particular, in the case of tumor EL-4 [12], that tumors of inbred animals have antigens analogous (or taken to be analogous by the autoimmune system) to normal transplantation antigens of animals of other lines [15, 18]. The presence of a strong alloantigen on a syngeneic tumor ought evidently to lead to its rejection. Evidently if tumor growth nevertheless takes place, the host responds insufficiently strongly to its "allo"-antigens. We suggest that changes in the region of antigens of the principal histocompatibility complex are a characteristic feature of widely different tumors, as one of the factors promoting tumor autonomy. The immunologic response to these antigens may give cross reactions with the individual's own transplantation antigens, including those represented on erythrocytes. Serologic data not contradicting this last hypothesis are beginning to appear in the literature [4, 11]. Research in this direction is continuing.

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