CYTOTOXIC ANTIBODIES AGAINST TUMOR CELLS IN THE BLOOD OF INTACT C3H MICE

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UDC 616-006-092.9-07:616.15-097.5

Cytotoxic antibodies against mammary gland carcinoma cells, L cells, and hepatoma 22a cells were found in the blood serum of male and female C3H/He and C3Hf mice over 8 months old. Similar antibodies were found in the serum of young and old BALB/c mice but were not found in C57BL/6 mice. The cytotoxic activity of the serum against mammary gland carcinoma cells was completely abolished by exhaustion with kidney tissue from syngeneic and allogeneic animals.

KEY WORDS: natural antibodies; cytotoxic antibodies.

Burnet's hypothesis of immunologic surveillance logically presupposes the presence of cytotoxic antibodies and lymphocytes directed against various spontaneous tumors in the blood of animals. In fact, a number of recently published papers show that so-called natural antibodies and cytotoxic lymphocytes, directed against certain tumors, are constantly present in the blood of many experimental animals and of man [3, 5, 7, 9, 10, 14]. The effect of such natural immunity on the rate of growth of lymphoid tumors has been clearly established in another investigation [16].

The object of the present investigation was to study natural cytotoxic antibodies in the blood of C3H mice directed against cells of a syngeneic primary mammary gland tumor.

EXPERIMENTAL METHOD

Samples of blood from an average of 10 mice of lines C3H/He, C3Hf, BALB/c, and C57BL/6 were obtained by decapitation of the animals. The serum was inactivated by heating to 56°C for 30 min. The cytotoxic reaction with freshly obtained rabbit complement (diluted 10 to 15 times) was carried out by the method published previously [1]. The activity of all sera (in a dilution of 1 : 10) was expressed in relative units compared with the activity of serum from 2-month-old male C3Hf mice, which produced lysis of 25% of target cells in the presence of complement. Absorption of cytotoxic antibodies was carried out on the following tissues: spleen cells from C3Hf: BALB/c mice (infected with Rauscher's virus) and of AKR mice (with progressive Gross' leukemia) in the proportion of 10^8 cells to 1 ml serum; washed homogenates of kidneys, liver, and spontaneous mammary gland tumors of C3H mice in the ratio of 1 g tissue to 1 ml serum. The following target cells were used: 1) mammary gland tumor cells MMT1 of C3H mice subcultured *in vitro*, 2) nonmalignant (L) and malignant (LS) sublines of L cells (fibroblasts of C3H mice subcultured *in vitro*), 3) hepatoma 22a cells from C3HA mice subcultured *in vitro*, and 4) embryonic fibroblasts of C3Hf mice. For each group of the animals at least three samples of serum were tested. Each experiment involving exhaustion of the sera was repeated at least three times. The pooled results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

Data on the cytotoxic activity of sera obtained from C3H/He mice, its factor-deficient subline C3Hf, and lines BALB/c and C57BL/6, against MMT1 cells are given in Fig. 1. They show that the blood of male and female C3H/He and C3Hf mice over the age of 8 months contains detectable antibodies giving a clear cytotoxic reaction with complement against mammary gland tumor cells. They were also found in old female C3H/He mice with spontaneous mammary gland

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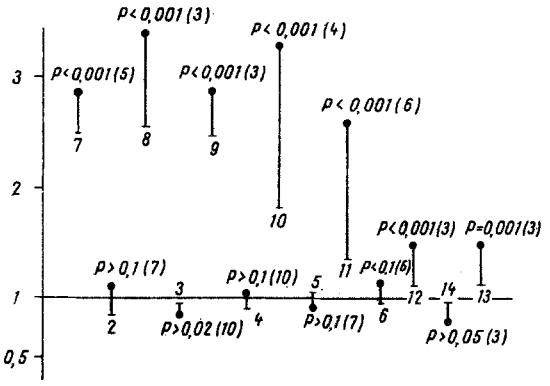

Fig. 1. Cytotoxic activity of sera of C3Hf, C3H/He, BALB/c, and C57BL/6 mice of different sexes and ages against MMT1 cells. 2) ♂C3Hf aged 2-3 months; 3) ♂ C3H/He aged 2-3 months; 4) ♀C3H/he,2-3 months; 5) ♂C3Hf,3-4 months; 6) ♀C3H/He, 3-4 months; 7) ♂C3Hf, 9-14 months; 8) ♀C3Hf, 9-14 months; 9) ♂ C3H/He, 9-14 months; 10) ♀ C3H/He, 9-14months; 11) ♂C3H/He with spontaneous mammary gland tumors; 12) ♂BALB/c, 2-3 months; 13) ♂ BALB/c, 9-14 months; 14) ♂ C57BL/6 9-14 months. The activity of all sera is indicated in relative units compared with the activity of sera of male CH3f mice aged 2-3 months, taken as 1 (along ordinate). Numbers in parentheses denote number of samples of sera tested.

TABLE 1. Cytotoxic Action of Sera of Old C3H/He Mice on Hepatoma 22a Cells, L and LS Cells, and Embryonic Fibroblasts (M ± m)

Target cells	Number of living cells remaining after treatment with C3H/He mouse serum		P
	not over 4 months old	over 8 months old	
Hepatoma 22a	83,7±6,7 (7)	53,8±4,7 (7)	<0,01
L	85,5±8,2 (4)	57,2±7,0 (7)	<0,05
LS	72,1±2,0 (4)	45,5±7,3 (7)	<0,05
Embryonic fibroblasts C3Hf	92,4±2,4 (3)	92,8±2,2 (13)	

Legend: Number of samples of sera tested shown in parentheses.

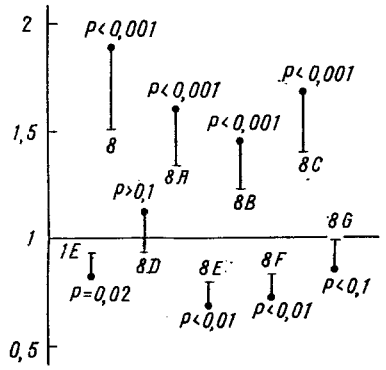

Fig. 2. Ability of tissues of different mouse organs to exhaust cytotoxic activity of C3Hf mouse sera. 1) Serum of C3Hf males aged 2-3 months; 8) Serum of C3Hf female mice over 8 months old. Activity of sera given in relative units compared with activity of sera of C3Hf males aged 2-3 months, taken as 1 (along ordinate). Letters denote tissues taken for exhaustion: A) Spleen cells from C3Hf mice; B) spleen cells from BALB/c mice infected with Rauscher virus; C) spleen cells of AKR mice with progressive leukemia; D) liver tissue of C3Hf mice; E) kidney tissue of C3Hf mice; F) kidney tissue of C57BL/6 mice; G) tissue of spontaneous mammary gland tumor of C3H/He mice.

tumors. Antibodies against MMT1 cells were found in lower concentrations in the blood of young and old BALB/c mice also, but were not present in C57BL/6 mice, even in old animals.

The serum of old C3H/He mice had a cytotoxic action on subcultured strains of other types of cells, namely on malignant and nonmalignant sublines of L cells and also on cells of hepatoma 22a. The serum had no such action, however, on embryonic fibroblasts (Table 1).

The experiments to study exhaustion of the sera of the old C3Hf mice showed that antibodies against MMT1 cells were completely absorbed on kidney tissues of syngeneic and allogeneic animals, and were absorbed to a large degree by liver tissue and tissues of spontaneous mammary gland tumors of C3H/He mice (Fig. 2). Spleen cells of intact mice and also of mice infected with Rauscher and Gross virus did not absorb the antibodies.

Serum from young CH3f mice also contained antibodies of the same specificity as those in the serum of the old animals, but in low concentration, for the sera of the young mice, exhausted with liver tissue, lost the traces of whatever weak activity they had possessed (Fig. 2).

It follows from the results given above that much of the cytolytic activity of the sera of the C3H mice was aimed against kidney and liver tissues or, perhaps, against virus antigens settling in these organs (not cross-reacting with Rauscher and Gross virus).

The preliminary results of determination of the activity of the sera of old C3H mice exhausted with kidney tissue showed that the activity of these sera against L-cells was not completely lost, whereas against MMT1 cells it disappeared altogether. Evidently the sera of old C3H mice are immunologically polyvalent, some of their activity being directed against tumor antigens. This hypothesis is confirmed by investigations showing that the serum of intact animals and man may contain antibodies directed against antigens of various viruses [6, 8, 13], against neoantigens of certain tumors [7, 9, 10], and against autoantigens of various tissues [2, 11, 12, 15]. The accumulation of factual material in this field and its systematic classification will provide a solid experimental foundation for Burnet's hypothesis of immunologic surveillance and will also enrich our knowledge of autoimmune processes.

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RESISTANCE OF RATS VACCINATED WITH BCG TO TUMOR GROWTH AFTER ADMINISTRATION OF CYCLOPHOSPHAMIDE AND DESENSITIZATION

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UDC 616-006.092:[615.371:576.852.211

Acceleration of growth of tumors was shown to take place after implantation of tumor cells not only in the early stages of sensitization of the animal with BCG (previously published data), but also in the late stages. Cyclophosphamide abolished this effect, but desensitization carried out after administration of cyclophosphamide restored it. It is concluded from the data on the connection between allergy to tuberculin and the state of the mechanisms of immunologic protection of the animal that tumor growth can be stimulated by procedures abolishing the state of inhibition of immunologic mechanisms.

KEY WORDS: Allergy; desensitization; cyclophosphamide; tumors.

BCG vaccine, in conjunction with other methods of treatment and, in particular, with chemotherapy, is widely used in clinical oncology. However, according to some investigators [12, 13] no convincing evidence of its beneficial effect on antitumor resistance has yet been obtained. Since the functional capacity of the immunologic system of the body is reduced in the presence of malignant neoplasms, the view has been expressed that there is no future for active immunotherapy in clinical oncology [6]. Attempts have recently been made to use in oncology methods based on removal of the components of reactivity which adversely effect the immunologic defence of the body [2, 11].

At the same time it has been shown that the resistance of the organism to tumors is always higher in the late stages after administration of BCG either per se or as a component of Freund's complete adjuvant than in the early stages (at the height of development of allergy), and may exceed its initial level [4]. The resistance of the body to tumors, in the presence of the immunologic reaction to BCG vaccine, can also be raised by removal of the allergic component of reactivity [2]. This can be explained on the grounds that as a result of desensitization the ability of the organism to carry out protective immunologic reactions is increased [1, 5, 9, 10].

However, it is not yet known whether the resistance of the body is increased in the late stages after administration of BCG vaccine as a result of the development of malignant cells which have already appeared, or what importance must be attached to the fact that during the immunologic response to BCG the animal was subjected to the action of cytotoxic drugs and, in particular, cyclophosphamide. These are important problems in connection with metastasization and recurrence of malignant neoplasms. We likewise have no information on whether the danger of immunologic potentiation of tumor growth exists when the components of reactivity adversely influencing immunologic protective reactions are excluded.

The investigation described below was carried out to study these matters.

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0007-4888/79/8705-0485$07.50