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PRODUCTION OF MONOCLONAL ANTIBODIES TO Lyt-2,3 ANTIGEN

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Mouse Lyt-2 and Lyt-3 antigens are membrane markers of T cells which appear in the course of their maturation in the thymus, are expressed in T killers and T suppressors, and are essential for the realization of their functions [4]. In particular, monoclonal antibodies (MCA) against Lyt-2,3 antigens block the cytotoxic action of T killers [14] and inhibit proliferation of T cells in mixed lymphocyte culture [6]. Lyt-2 and Lyt-3 molecules, with molecular weights of 34-38 and 30 kilodaltons respectively, are expressed on the surface of the same cells, their chains are covalently linked, and they form mono-, di-, and tetramers [11]. Several hybridomas producing MCA against Lyt-2 antigen are known [5, 7, 9]. Lyt-3 antigen has received less study.

This paper describes the production and properties of MCA against Lyt-2,3 antigens.

EXPERIMENTAL METHOD

Mice of lines CBA, BALB/c, C57BL/6, DVA/2, and AKR were obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, and of lines SJL and C58 from the Research Laboratory of Experimental Biology and Medicine, Academy of Medical Sciences of the USSR. AKR mice were immunized by a single intravenous injection of $2 \cdot 10^7$ thymocytes from CBA mice. Seven days later, $5 \cdot 10^7$ spleen cells of the immune mice were hybridized by means of 50% polyethylene-glycol (mol. wt. 1500 daltons, from Schuchardt, West Germany) with 10^7 mouse myeloma P3-X63-Ag 8.653 (X63) cells [3]. After hybridization the cells were transferred in DMEM medium with 10% embryonic calf serum, 4 mM L-glutamine (all from Flow Laboratories, England), 100 units/liter gentamicin (from Farmakhim, Bulgaria), 10^{-4} M hypoxanthine, $4 \cdot 10^{-7}$ M aminopterin, and $1.6 \cdot 10^{-5}$ M thymidine (all from Sigma, USA), into 96-well plates (3040, from Falcon, USA), seeded beforehand with peritoneal macrophages (10^4 cells per well). The presence of antibodies in the culture fluid (CF) was determined 10-14 days later in a two-stage complement-dependent cytotoxicity test [2], using rabbit complement (from Cedarline, Canada). During mass treatment $5 \cdot 10^7$ cells were incubated in 5 ml of complement (1:10) for 1 h at 37°C. Dead cells were removed by centrifugation in a Ficoll—Hypaque density gradient. Treatment of the cells with anti-Thy-1,2 serum (from Searle, England), diluted 1:20, was used as the positive control.

The indirect immunofluorescene test was carried out by incubating 10^6 cells in 50 µl of CP and, after washing, in 50 µl of rabbit antimouse antibodies labeled with fluorescein isothiocyanate (FITC N. F. Gamaleya Institute of Epidemiology and Microbiology). Fluorescence was recorded on a 50-H flow cytometer (Ortho, USA).

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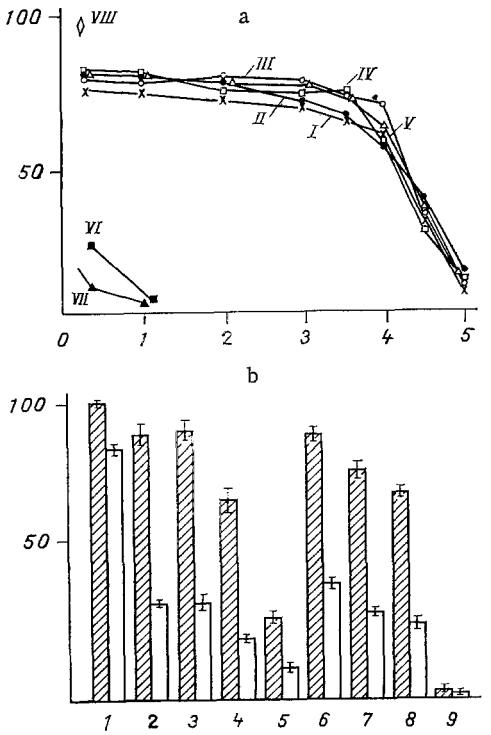

Fig. 1. Cytotoxic action of MCA F9 on thymocytes of different strains of mice (a) and on lymphoid cells of BALB/c mice (b). Abscissa: a) $\log_{10}$ of dilution of F9; b) source of cells; ordinate, cytotoxic index (in %). a: Thymocytes from CBA (I), SJL (II), DBA (III), BALB/c (IV), C57BL/6 (V), AKR (VI), and C58 (VII) mice, positive control with anti-Thy-1,2-serum on thymocytes of the same lines (VIII); b: reaction of anti-Thy-1,2-serum (shaded columns) and MCA F9 (unshaded columns) with cortical thymocytes (1), medullary thymocytes obtained with the aid of A30 (2), or injection of hydrocortisone *in vivo* (3), with lymph node (4) and spleen cells (5), with lymph node (6) and spleen T cells (7), with lymph node cells treated beforehand, in the presence of complement, with normal mouse serum (8) or anti-Thy-1,2-serum (9).

Radioimmunoassay was done by the method in [1]. Mouse MCA L3E8 against L-chains of rat immunoglobulin were used as ^{125}I-labeled antibodies. The results were expressed as the ratio of activity of the label in the experimental and control samples (treatment of UF of X63 myeloma and ^{125}I-MCA L3E8). The rat MCA against Lyt-1 and Lyt-2 antigens were provided by R. G. Vasilov [9].

T cells of the lymph nodes and spleen were separated from B cells by the method described previously [12]. Cortical and medullary thymocytes were separated with the aid of groundnut agglutinin A30 (from Boehringer, West Germany) [8]. The purity of the fractions of cortical thymocytes ($A30^+$) and medullary thymocytes ($A30^-$) was tested by a fluorescence method, by treating the cells with A30, conjugated with FITC. The degree of purity was not less than 95%. Medullary thymocytes also were obtained from mice treated intraperitoneally with 2.5 mg of hydrocortisone 48 h before the experiment.

EXPERIMENTAL RESULTS

CF from wells in which growth of hybridomas was observed were tested for the presence of antibodies in the cytotoxic test with thymocytes from CBA mice. A positive NATF9 culture was cloned twice by the terminal dilutions method at the rate of one cell per well. MCA secreted by clone NATF9.9 (abbreviation to F9), judging by the results of gel-filtration on Sephadex G-200, belonged to the IgM class. To elucidate the nature of the antigen revealed by MCA F9, their reactivity against mouse thymocytes of different strains was investigated. As Fig. 1 shows, MCA F9 in the presence of complement killed 75-80% of thymocytes of CBA, BALB/c, C56BL/6, DBA/2, and SJL mice in a dilution of CF of 1:10,000, but did not kill any thymocytes of AKR and C58 mice even in a dilution of 1:10.

The study of the distribution of F9 antigen in other lymphoid tissues showed that the number of $F9^+$ cells increased during an increase in the number of T cells from 10 to 29% in the spleen and from 20 to 38% in the lymph nodes, and fell to zero after removal of the T cells by anti-Thy-1,2-serum (Fig. 1). These results indicate that F9 antigen is expressed only on T cells. It will also be clear from Fig. 1b that the number of $F9^+$ cells decreased during maturation of the thymocytes: from 85% in the cortical thymus to 32% in the medullary thymus and peripheral T lymphocytes.

During analysis of the cells by flow cytometry histograms of fluorescence were obtained, which were bimodal in character for thymocytes and T cells from lymph nodes, but asymmetrical in shape for splenic T cells (Fig. 2). Counting the number of fluorescent cells showed that F9 antigen was present on 80% of thymus cells, on 39% of lymph node T cells, and on 42% of

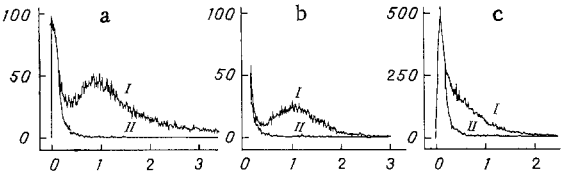

Fig. 2

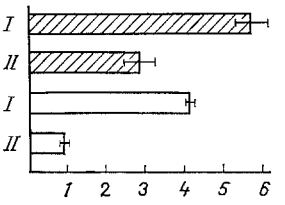

Fig. 3

Fig. 2. Distribution of thymocytes (a) and T cells of lymph nodes (b) and spleen (c) by intensity of fluorescence, measured by flow cytometry. Cells treated with MCA F9 (I) or control CF (II). Abscissa, intensity of fluorescence (in relative units); ordinate, number of cells.

Fig. 3. Expression of Lyt-1 (I) and Lyt-2 (II) antigens on lymph node cells of BALB/c mice after removal of cells reacting with MCA F9 or with control CF (shaded columns). Abscissa, binding index of MCA against rat immunoglobulin L-chains labeled with ^{125}I. Results of two experiments (M ± m).

splenic T cells. The data of immunofluorescence coincided with the results of the cytotoxicity test for thymocytes, but were somewhat higher than the same results for T cells of the lymph nodes and spleen. The distribution of F9 antigen obtained in lymphoid tissues corresponded to the distribution of Lyt-2 and Lyt-3 antigens [10].

For the direct study of binding of F9 antigen with Lyt-1 and Lyt-2, lymph node cells from BALB/c mice were treated with CF of F9 with complement, and after removal of the dead cells, expression of antigens Lyt-1 and Lyt-2 was determined on the remaining surviving cells by radioimmunoassay. It will be clear from Fig. 3 that removal of the $F9^+$ cells led to complete abolition of binding of anti-Lyt-2 MCA while preserving Binding of anti-Lyt-1 with MCA to the extent of 70% compared with control treatment of the cells. Lyt-2 antigen was thus present only on $F9^+$ cells, whereas most lymphocytes carrying Lyt-1 did not contain F9 antigen.

Since F9 antigen is represented in the largest numbers of thymus cells and cortical thymocytes (Fig. 1), the distribution of alleles of five antigens (Thy-1, TL, Lyt-1, 2, and 3) [13], expressed on cortical thymocytes, among the lines of mice was compared with the ability of thymocytes of these lines to react with the MCA obtained. The results in Table 1 show that AKR anti-CBA MCA can potentially react with Thy-1,2, Lyt-1,1, and Lyt-3,2 antigens, but not with Lyt-2 antigen, whose Lyt-2,1-allele is identical in AKR and CBA mice. The positive reaction of the same MCA with thymocytes of C57BL/6 and BALB/c mice (Lyt-$1,2^+$) excludes any possible participation of antibodies against Lyt-1,1 antigen and, consequently, it disproves any binding of F9 with Lyt-1 antigen. The difference between F9 antigen and Thy-1 and Lyt-1 antigens also follo-s from the fact that F9 MCA do not react in the cytotoxic test with thymoma EL4 cells of C57BL/6 mice with the Thy-$1,2^+$, Lyt-$1^+2^-3^-$ phenotypes.

It follows from the data described above that the antigen detected by F9 AKR anti-CBA MCA is neither an autoantigen (absent on AKR thymocytes) nor the universal antigen of T cells Thy-1, nor Lyt-1 antigen, nor Lyt-2 antigen. Since F9 antigen is expressed on the same T

TABLE 1. Distribution of Alleles of Antigens Expressed on Cortical Thymocytes and Reactivity of F9 MCA among Mouse Strains

Source of cells (strain of mice)	Alleles of antigens					
	Thy-1	TL	Lyt-1	Lyt-2	Lyt-3	F9
C57BL/6	2	—	2	2	2	+
BALB/c	2	2	2	2	2	+
DBA/2	2	2	1	1	2	+
CBA	2	—	1	1	2	+
SJL	2	1, 2, 3, 5	2	2	2	+
C58	2	1, 2, 3, 5	2	1	1	—
AKR	1	—	2	1	1	—

cells as Lyt-2 antigen, the only suggestion which remains is that it is Lyt-3 antigen. This hypothesis is confirmed by the fact that presence or absence of reaction of F9 MCA (Table 1) correlates precisely with the presence or absence of Lyt-3,2 antigen, but not of other antigens, on the thymocytes of mice of this particular line.

Since two groups of determinants were discovered [7] on Lyt-2 antigen with the aid of MCA it can be tentatively suggested that the same groups of determinants exist on Lyt-3 antigen, which is linked with Lyt-2. In that case the F9 MCA which we obtained evidently reveal a determinant of the second group of Lyt-3 antigen, expressed on a relatively smaller proportion of T cells of lymph nodes and spleen.

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CHANGES IN HOST RESISTANCE TO CANCER DURING CHEMICAL CARCINOGENESIS

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After a single injection of a chemical carcinogen (9,10-dimethyl-1,2-benzanthracene — DMBA) into noninbred rats a long time elapses before the appearance of a tumor. The duration of the latent period depends on the dose and site of administration of the carcinogen. The writer has shown [1, 2] that after peroral administration of 20 mg DMBA to rats weighing 90-100 g it is 5-9 months, whereas after subcutaneous injection of 0.6-4 mg into rats of the same weight it is only 2-4 months.

The aim of this investigation was to study the resistance of rats at different stages of growth of a tumor induced by subcutaneous injection of DMBA as reflected in the successful taking of tumors with different levels of transplantability.

EXPERIMENTAL METHOD

An injection of 4 mg of DMBA in 0.2 ml of peach oil was given subcutaneously into the left side of female rats weighing 90-100 g. Three months later, 30 animals from this group were selected, in which foci of induration no bigger than a lentil (tumor anlage) could be

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