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DEMONSTRATION OF A DIFFERENTIAL ANTIGEN OF ACTIVATED T AND B LYMPHOCYTES (ACA-1) ON TUMOR CELLS

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The view has recently been formed in immunology that differentiation of cells is accompanied by changes in the antigenic properties of their cell surfaces [2, 8]. Antigenic markers of intact T and B lymphocytes and of their subpopulations have now been discovered [1, 6]. After stimulation of lymphocytes by monspecific mitogens or antigens, several workers have found antigenic markers of the stimulated lymphocytes [3, 7, 11, 13, 14]. The writers showed previously that a special ACA-1 antigen (activated cell antigen), which is not found on intact T and B lymphocytes, is present on the surface of activated T and B lymphocytes [2-4, 5].

The aim of this investigation was to demonstrate this antigen on intensively proliferating tumor cells of different histogenetic origin.

EXPERIMENTAL METHOD

Mice of both sexes and of the C57BL/6 $(H\text{-}2)^b$, $C57BL/10_{Sn}$ $(H\text{-}2^b)$, C3H $(H\text{-}2^k)$, A/Sn $(H\text{-}2^a)$, BALB/c $(H\text{-}2^d)$, CBA $(H\text{-}2^k)$, (CBA × C57BL/6) F_1 $(H\text{-}2^{k/b})$ lines, weighing 18-20 g, were obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR.

Ascites forms of tumors Sa-1 $(H\text{-}2^a)$, MCh-11 $(H\text{-}2^b)$, and EL-4 $(H\text{-}2^b)$ were generously provided by B. D. Brondz, and an ascites form of tumor AG-22 by Yu. A. Rovenskii (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR). Serial passages of the tumors were carried out every 8-13 days by intraperitoneal injections of tumor cells into intact recipients ($5 \cdot 10^6$-$10 \cdot 10^6$ cells per mouse). C3H mice were used for passage of hepatoma AG-22, A/Sn mice for sarcoma Sa-1 and $C57BL/10_{Sn}$ or C57BL/6 mice for T lymphoma EL-4 and tumor MCh-11.

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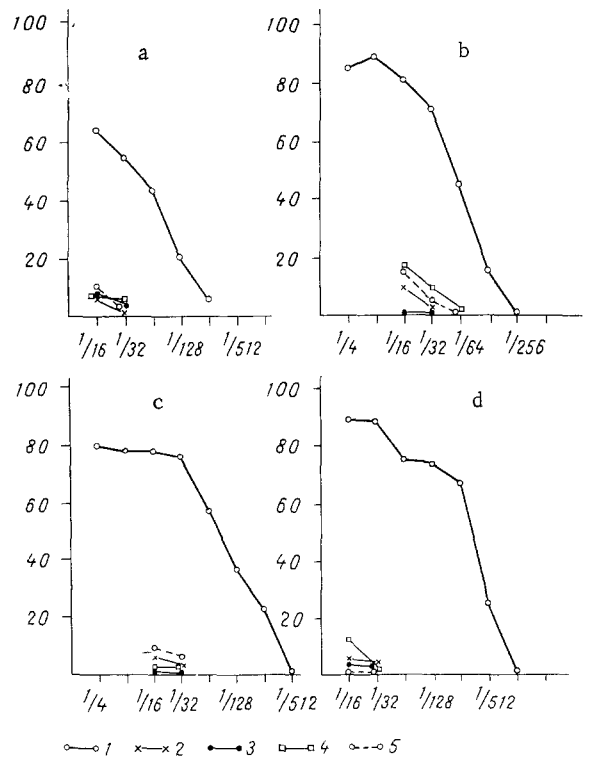

Fig. 1

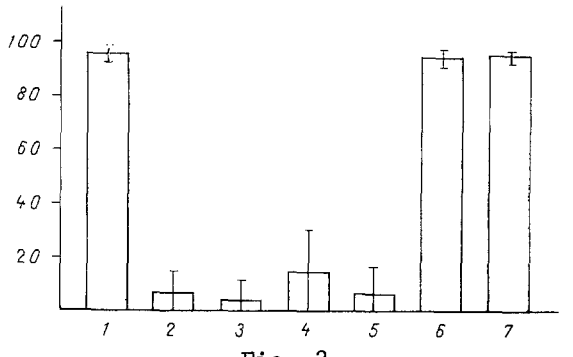

Fig. 2

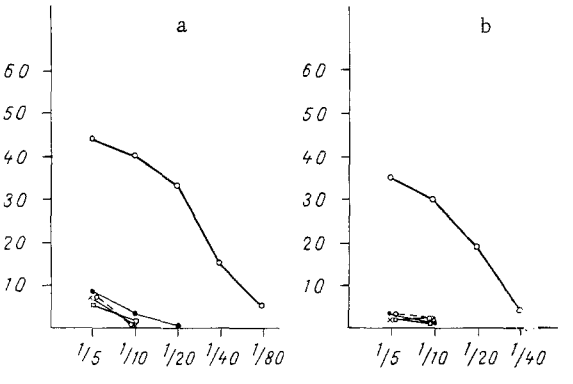

Fig. 3

Fig. 1. Cytotoxic activity of anti-ACA-1 against tumor cells. Abscissa, dilutions of anti-ACA-1; ordinate, CTI (in %). Target cells for cytotoxic action of anti-ACA-1: a) Sa-1 (results of six experiments); b) AG-22 (results of eight experiments); c) EL-4 (results of eight experiments); d) MCh-11 (results of five experiments). 1) Anti-ACA-1 before absorption; 2) anti-ACA-1 after absorption by Sa-1; 3) after absorption by AG-22; 4) after absorption by EL-4; 5) after absorption by MCh-11. Additional absorption carried out at the rate of $3.3–10^8$ tumor cells to 1 ml anti-ACA-1 or $4.5\text{-}10^8$ intact cells (lymphocytes or liver cells) to 1 ml of anti-ACA-1.

Fig. 2. Effect of anti-ACA-1 on AFC. Ordinate, % inhibition of AFC (results of 10 experiments). 1) Anti-ACA-1 before absorption; 2) anti-ACA-1 after absorption by Sa-1; 3) after absorption by AG-22; 4) after absorption by EL-4; 5) after absorption by MCh-11; 6) after absorption by intact lymphocytes; 7) after absorption by liver cells. AFC detected in spleens of (CBA x C57BL/6)F_1 mice 4 days after intravenous immunization with SRBC. Dilution of anti-ACA-1 was 1:250.

Fig. 3. Cytotoxic activity of anti-ACA-1 against T_{act}. a) Target cells CBA-anti-C57BL/6 T_{act}; b) target cells CBA-anti-BALB/c T_{act}. Results of eight experiments shown. Remainder of legend as to Fig. 1.

Antibody-forming cells (AFC) producing 19S-antibodies against sheep's red blood cells (SRBC) were detected by the method in [10]. T lymphocytes activated by allogeneic transplantation antigens (T_{act}) were obtained by the method in [12]. CBA-anti-C57bl/6 T_{act} and CBA-anti-BALB/c T_{act} were used.

The method of preparing and testing the rabbit antiserum against ACA-1 (anti-ACA-1) was described previously [2, 4, 5]. Rabbits were immunized with T_{act}, among which 30-60% of the cells consisted of medium-sized lymphocytes and blast cells; the resulting immune serum was absorbed by erythrocytes, serum, and thymus, spleen, and lymph node cells from intact mice. The action of antiserum on T_{act} was assessed in the complement-dependent lymphocytotoxic test [3], and its action on AFC by the test of reduction of the number of AFC after treatment of a suspension of spleen cells from mice immunized with SRBC by antiserum with rabbit complement (RC) [4]. In the latter case depression of AFC was calculated as a percentage by the formula $(a - b)/a \cdot 100\%$, where a is the number of AFC after treatment of the cells with RC, and b the number of AFC after treatment with antiserum and RC.

The action of antiserum on tumor cells was demonstrated in the complement-dependent cytotoxic test by the following method. To 0.15 ml of the corresponding dilution of antiserum, 0.05 ml of thrice washed cells ($1 \cdot 10^6$ cells/ml) and 0.05 ml of nontoxic rabbit serum (the source of complement) were added. The mixture was incubated for 45 min at 37°C, after which 0.25 ml of 0.1% trypan blue solution with 0.1% eosin solution was added and the percentage of dead cells was counted after stainig with dyes. For each dilution of antiserum the cytotoxic index (CTI) was calculated by the formula $(a - b)/(100 - b) \cdot 100\%$, where a is the percentage of dead cells on treatment with antiserum and RC, and b the percentage of dead cells after treatment with RC alone. Binding of rabbit antisera with target cells also was estimated in the indirect immunofluorescence test [9]. Fluorescein-labeled donkey antibodies against rabbit IgG, obtained from K. L. Shakhanina (N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR), were used for this purpse.

EXPERIMENTAL RESULTS

The results of 20 experiments to assess the action of anti-ACA-1 on cells of different tumors before and after absorption by tumor cells are shown in Fig. 1. Before absorption, anti-ACA in a dilution of 1:4-1:16 produced lysis of 64% of Sa-1 cells, 89% of AG-22 cells, 80% of EL-4 cells, and 89% of MCh-11 cells. Additional absorption of anti-ACA-1 by Sa-1, AG-22, MCh-11, and EL-4 tumor cells considerably reduced its cytotoxic activity against all tumor cells tested (Fig. 1), whereas absorption with intact lymphocytes or liver cells of intact mice did not alter the cytotoxic activity of anti-ACA-1 (the results are not shown). In the indirect immunofluorescence test anti-ACA-1 induced fluorescence of 58% of Sa-1 cells, 84% of AG-22 cells, 80% of MCh-11 cells, and 83% of EL-4 cells (data not shown). It must be noted that anti-ACA-1 virtually did not react at all with intact lymphocytes in the cytotoxic test [2, 5] or in the indirect immunofluorescence test.

It was postulated on the basis of these results that ACA-1 is present on actively proliferating cells of the tumors tested (just as also on activated T and B lymphocytes). The data in Figs. 2 and 3 confirm this hypothesis. Anti-ACA-1 depressed the number of AFC by 95%. Additional absorption of anti-ACA-1 by Sa-1, AG-22, MCh-11, and EL-4 tumor cells reduced its inhibitory action by 86-100%. Absorption by intact lymphocytes or by liver cells of intact mice did not affect the ability of anti-ACA-1 to inhibit AFC (Fig. 2). Absorption by tumor cells similarly reduced the cytotoxic activity of anti-ACA-1 against CBA-anti-C57BL/6 T_{act} and CBA-anti-BALB/c T_{act}. In dilutions of 1:5-1:10 anti-ACA-1 killed 44% of CBA-anti-C57BL/6 T_{act} and 35% of CBA-anti-BALB/c T_{act}. Additional absorption by Sa-1, AG-22, MCh-11, and EL-4 tumor cells reduced its cytotoxic activity in these same dilutions by 82-100% for CBA-anti-C57BL/6 T_{act} and by 66-98% for CBA-anti-BALB/c T_{act} (Fig. 3). After additional absorption of anti-ACA-1 by intact lymphocytes or by liver cells of intact mice, its cytotoxicity was virtually unchanged (data not shown).

It can be concluded from these results that ACA-1 can evidently be demonstrated not only on activated normal T and B mouse lymphocytes, but also on actively proliferating tumor cells of the lymphoid (EL-4) and nonlymphoid series (Sa-1, AG-22, MCh-11). It can be tentatively suggested that ACA-1 is a universal antigenic marker of proliferating cells.

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PREPARATION OF SECONDARY T KILLERS

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The solution to the problem of antitumor and transplantation immunity is directly bound up with the study of differentiation and mechanisms of action of cytolytic T lymphocytes (CTL). Several model systems have been developed for obtaining CTL *in vivo* and *in vitro* [1, 7, 8, 13]. On immunization *in vivo* mainly small and medium lymphocytes, which can be obtained from the spleen, lymph nodes, or peritoneal exudate on the 11th-12th days after injection of a tumor allograft [8], possess cytolytic activity. Conversely, in mixed lymphocyte culture (MLC), the peak of cytolytic activity is found on the 4th-5th days and the target cells (TC) destroy large lymphocytes and lymphoblasts [4]. On immunization *in vitro*, CTL proliferate in response to a single antigenic stimulus, whereas *in vivo* the action of the antigenic stimulus continues until rejection of the allograft.

This paper decribes an attempt to reproduce *in vitro* the conditions corresponding to differentiation of CTL *in vivo*.

EXPERIMENTAL METHOD

Inbred BALB/c ($H\text{-}2^d$) and C3H ($H\text{-}2^k$) mice aged 8-12 weeks were used.

CTL were obtained in MLC by the method in [9]. Reacting spleen cells of BALB/c mice, in a concentration of $2\cdot10^6$ cells/ml, and stimulating spleen cells in concentration of $1\cdot10^6$ cells/ml, irradiated in a dose of 1000 R (10 Gy), from C3H mice were cultured in RPMI-1640 medium containing 15% embryonic calf serum(ECS), $3\cdot10^{-5}$ M mercaptoethanol, $2\cdot10^{-3}$ M L-glutamine, $5\cdot10^{-3}$ M HEPES, and penicillin and streptomycin each in a concentration of 100 units/ml medium, in Sani Glas flasks in an atmosphere of 5% CO_2 at 37°C for 5, 8, and 12 days. On the 5th day the cells were sedimented by centrifugation and transferred to freshly prepared medium without addition of mercaptoethanol.

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