INDUCTION OF XENOGENEIC ANTIBODIES AGAINST KILLER T CELLS OF MICE IMMUNE TO H-2 ANTIGENS

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UDC 612.112.94.017.4

KEY WORDS: antisera against killer T cells; idiotypic receptor; differential antigens.

Specific antibodies against idiotypic determinants of receptors and against differential antigens of T cells have been obtained during immunization of syngeneic [5], allogeneic [8] and xenogeneic [9, 11, 12] animals with mouse and rat T cells. However, the question of the presence and nature of surface antigenic structures characterizing individual subpopulations of T lymphocytes and, in particular, of killer T cells, and also of their relations to the specific function of these cells, has not yet been studied.

In the investigation described below rabbit antisera were obtained against immune mouse killer T cells, induced by allogeneic cells in vivo and in vitro, and the effect of these antibodies was studied on the specific function of the killer T cells by attempting to identify, in particular, which determinants of the lymphocyte surface may be associated with this effect.

EXPERIMENTAL METHOD

To obtain cytotoxic peritoneal lymphocytes (CPL) in vivo, CBA (H-2^k), BALB/c (H-2^d), and AKR (H-2^k) mice were immunized intraperitoneally with 25×10^6 cells of an EL-4 ascites lymphoma maintained by passage through C57BL/6 (H-2^b) (abbreviated to B6) mice [6]. CBA mice also were immunized with cells of an L-1210 lymphoma, induced in DBA/2 (H-2^d) mice. On the 10th-12th day peritoneal exudate cells were freed from macrophages and remains of the tumor by absorption twice on plastic Petri dishes for 45 min each time, at 37°C, after which the suspension contained 90-95% of small lymphocytes; 2×10^8 of these cells from CBA mice were injected into rabbits in Hanks' solution by 5 intravenous injections at intervals of 20-30 days.

To induce cytotoxic cultural lymphocytes (CCL) in mixed cultures in vitro, lymph node cells from CBA or BALB/C mice were used as reacting cells, and spleen cells from B6 mice, irradiated in a dose of 1500 rad, as stimulating cells. A mixture of these cells in the ratio of 4:3 was incubated for five days at 37°C in an atmosphere containing 5% CO_2 in glass flasks ("Sany Glass") in RPMI-1640 medium with 10% embryonic calf serum, 2 mM L-glutamine, 5 mM HEPES buffer, 5×10^{-5} M 2-mercaptoethanol, and antibiotics. Immune lymphocytes were washed and enriched with lymphoblasts up to 70-90% by fractionation in a linear Ficoll gradient [10]. The rabbits were given 2.3×10^8 CBA CCL by three intravenous injections. Blood serum obtained on the 10th day after the last injection was inactivated and adsorbed by intact mouse tissues, mouse erythrocytes (three times) and blood serum, and, to remove antibodies against intact lymphocytes, with a mixture of mouse lymph node, spleen, and thymus cells (twice, 5×10^8 to 8×10^8 cells/ml serum) [4]. After absorption the serum was clarified (30,000 rpm, 30 min), poured out, and kept at −35°C. Antibodies were determined by a micromodification of the two-stage [1] or one-stage [4] cytotoxic test, using rabbit complement from "Cedarlane" in a dilution of 1:15, or of rabbit serum, chosen for nontoxicity, in a dilution of 1:20. The dilution of the serum at which the cytotoxicity was 20% was taken as its titer.

CPL and CCL in doses of 6×10^6 to 8×10^6 cells were treated with 0.1 ml antiserum in a dilution of 1:3 or 1:6 and with 0.1 ml complement for 1 h at 37°C, washed three times, counted, and equalized for number of living cells. The cytotoxic effect of CPL and CCL was determined in a microtest [2], using ^{51}CR-labeled mouse peritoneal macrophages [3] or EL-4 ascites lymphoma cells [7] as the target cells.

Laboratory of Immunochemistry and Diagnosis of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 7, pp. 62-64, July, 1980. Original article submitted July 17, 1979.

0007-4888/80/8907-0936$07.50

TABLE 1. Activity of Antisera in Cytotoxic Tests with Complement

Exhausted antisera against cells	Target cells				
	CBA anti-B6 CPL	BALB/C anti-B6 CPL	CBA anti-B6 CCL blasts	EL-4 lymphoma	Ehrlich's ascites carcinoma
CBA anti-B6 CPL	30 (1/8)†	0	0	0	0
CBA anti-B6 CCL	50—80 (1/8)	95 (1/16)	70 (1/16)	50—75 (1/32—1/64)	0
Mouse T lymphocytes*	85 (1/1024)	95 (>1/256)	80 (>1/128)	100 (>1/2048)	Not determined

Legend. *) Rabbit serum against mouse T lymphocytes from Cedarlane, Canada. †) Maximal cytotoxicity of serum (in % of killed cells). Titer of serum given in parentheses.

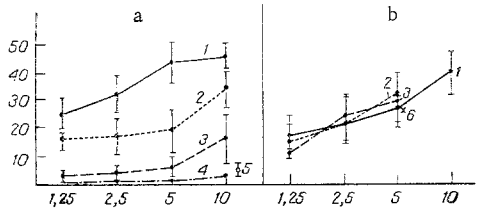

Fig. 1

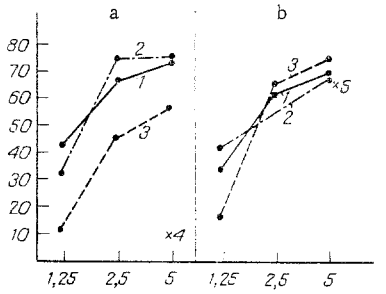

Fig. 2

Fig. 1. Effect of treatment of CBA anti-B6 CPL with sera against CPL and CBA anti-B6 CCL with (a) and without (b) complement on the cytotoxic effect on CBA (1) or B6 (2-6) macrophages. Abscissa, dose of immune lymphocytes ($\times 10^5$); ordinate, cytotoxic effect (in %). CPL untreated (1), treated with NRS (2), anti-CPL (3), anti-CCL (4), and anti-T (5), 5% anti-CPL present constantly in the culture medium (6). Vertical lines indicate mean error of 3-5 experiments.

Fig. 2. Effect of treatment of CBA anti-B6 CCL with sera against CPL and CBA anti-B6 CCL with (a) and without (b) complement on cytotoxic effect on B6 macrophages. CCL treated with NRS (1), anti-CPL (2), anti-CCL (3), anti-T (4), 5% anti-CCL constantly present in the culture medium (5). Remainder of legend as in Fig. 1.

In the control the cells were treated with normal rabbit serum (NRS) and absorbed with mouse erythrocytes and thymocytes and with complement. The statistical significance of differences was determined by Student's test.

EXPERIMENTAL RESULTS

Native unabsorbed anti-CPL and anti-CCL antisera were toxic against CBA mouse lymph node cells, killing 100% of these cells in a titer of more than 1: 2048. After absorption the sera became completely inactive in the cytotoxic test with complement against lymph node and spleen cells of intact CBA mice.

Exhausted anti-CPL serum proved to be toxic only against CBA anti-B6 CPL, against which they had been obtained, but not against other targets (Table 1). Conversely, anti-CCL killed cytotoxic cells of CBA mice and of mice of the "foreign" line – BALB/C anti-B6 CPL. Anti-CCL also killed T cells of lymphoma EL-4, but was nontoxic against tumor cells of nonlymphoid origin, such as Ehrlich's ascites carcinoma.

Preliminary treatment of the CPL with sera against CPL and CCL in the presence of complement inhibited the cytotoxic effect of the CBA CPL on B6 ($H\text{-}2^b$) macrophages (Fig. 1) or on EL-4 ($H\text{-}2^b$) cells. Serum against CPL had no inhibitory action on BALB/C anti-B6, AKR anti-B6, and CBA anti-DBA/2 lymphocytes, i.e., it was linearly and idiotypically specific (Fig. 3). Anti-CCL was more active. It inhibited the cytotoxic effect of CPL by 63-90%, whereas anti-CPL inhibited it by 25-55% (Fig. 1a). Anti-CCL serum, by contrast with anti-CPL, after treatment with complement, also inhibited the cytotoxic effect of CCL (Fig. 2a). Treatment of the killer cells with antisera but without complement did not affect their cytotoxic activity (Figs.

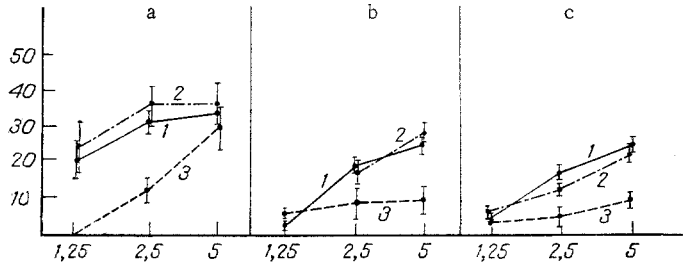

Fig. 3. Effect of treatment of BALB/C anti-B6 (a), AKR anti-B6 (b), and CBA anti-DBA/2 CPL (c) with sera against CPL and CBA anti-B6 CCL with complement on cytotoxic effect. CCL treated with NRS (1), anti-CPL (2), anti-CCL (3). Vertical lines indicate M ± m for one experiment. Remainder of legend as in Fig. 1.

1b and 2b). Serum against CBA anti-B6 CCL was able to inhibit the cytotoxic effect of CPL of the "foreign" BALB/C and AKR lines of mice, immune against allogeneic targets of the same haplotype (H-2^b) (see Fig. 3a, b) and of CPL of CBA mice, immune to DBA/2 (H-2^d) cells (see Fig. 3c).

Serum against CPL was thus less active as regards inhibition of the cytotoxic effect, but was more specific than anti-CCL. It acted only on those killer cells against which it had been obtained – CPL. Conversely, anti-CCL was more active, and its action was independent of the linear origin or immunologic specificity of the immune lymphocytes. It affected both small CPL and blast CCL. Evidently the action of serum against CPL is linked with the presence of antibodies against the idiotypic determinant and not against the surface marker of the killer T cells [11]. Meanwhile activity of the serum against CCL-blasts is evidently linked with the presence of antibodies against the stronger surface antigen of D-lymphocytes, which is present evidently also on cells of EL-4 lymphoma. The possibility cannot be ruled out that antibodies against the idiotypic determinant of killer T cells, to detect which the nonspecific surface determinants of the immune lymphocytes must be "screened" by antibodies against "blood" antigens [12], are also present in this serum. It is probable that during immunization of rabbits with mouse CCL the principal immunogeneic components are surface antigens of activated lymphocytes, the so-called ALA-antigens, which are found on immunization in an allogeneic mouse system [8]. As an approach to the study of regulation of the immune response it is interesting to examine the question of the connection between differential determinants of killer T cells and other subpopulations of T cells.

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