DETECTION OF A SPECIFIC DIFFERENTIAL ANTIGEN OF MOUSE T CELLS ACTIVATED BY ALLOGENEIC TRANSPLANTATION ANTIGENS

V. G. Nesterenko and Sh. G. Gruner

UDC 612.112.94.017.1+616.155.32-097.2

KEY WORDS: antigens; antibodies; activated lymphoid cells.

Data have recently been published to show that during stimulation of lymphocytes the antigenic properties of their surface are chang d and that activated lymphoid cells have special antigenic markers which distinguish them from intact lymphocytes [1, 3-5, 6].

The object of this investigation was to detect specific antigenic markers of mouse T lymphocytes activated by allogeneic transplantation antigens (T_{act}). The idea was to obtain, in a xenogeneic system, antibodies reacting only with T_{act}, and not with intact lymphoid cells.

EXPERIMENTAL METHOD

Male and female CBA ($H\text{-}2^k$), C57BL/6 ($H\text{-}2^b$), DBA/2 ($H\text{-}2^d$), BALB/C ($H\text{-}2^d$), C3H ($H\text{-}2^k$), and (CBA × C57BL/6)F_1 ($H\text{-}2^{k/b}$) mice, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, weighing 18-20 g, and (CBA × C3H·OH)F_1 ($H\text{-}2^{k/02}$) mice bred in our own animal house, were used.

T lymphocytes activated by allogeneic transplantation antigens (T_{act}) were obtained by the method described by Sprent and Miller [7]: 10^8 thymocytes from mice of one line were injected intravenously into allogenic mice or (CBA × C57BL/6)F_1 and (CBA × C3H·OH)F_1 hybrids, irradiated in a dose of 850 R, and 3 days after the injection, cell suspensions were prepared from the recipients' spleens. These cell suspensions contained 30-60% of transformed lymphocytes. To obtain antiserum against T_{act} ($AT_{act}S$), rabbits were immunized intravenously with $5 \cdot 10^8$ T_{act} CBA-anti(CBA × C57BL/6)F_1. The immunization was carried out three times with intervals of 1 month between injections. Seven days after the last immunization the rabbits were exsanguinated. The serum thus obtained was absorbed with the liver, erythrocytes, and serum of the mice, and also with thymus, lymph nodes, and spleen cells of intact mice. The absorption was continued until complete disappearance of activity of the sera in the hemagglutination test with mouse erythrocytes, in the gel precipitation test with serum of intact mice, and in the cytotoxic test with intact lymphocytes. Altogether five batches of $AT_{act}S$ were obtained. Activity of the $AT_{act}S$ was tested in the lymphocytotoxic test, the viability of the cells being determined by means of trypan blue [1].

CBA-anti-C57BL/6 and C57BL/6-anti-CBA antilinear sera, obtained by the usual method [2], and rabbit antiserum against mouse lymphocytes (ALS) and against mouse T lymphocytes (ATS) also were used. The specificity and high activity of the antilinear sera and ATS were verified in the cytotoxic test and by their effect on cells producing antibodies against sheep's red blood cells [2]. In these tests antilinear CBA-anti-C57BL/6 serum reacted with C57BL/6 cells, but did not act against CBA cells, whereas C57BL/6-anti-CBA serum reacted with CBA cells, but did not react with C57BL/6 cells. In the cytotoxic test ATS caused death of 100% of thymus cells, 60% of lymph node cells, and 35% of spleen cells, but had no effect on bone marrow cells or antibody-forming cells.

Special additional absorption of $AT_{act}S$ was carried out at room temperature for 1 h, taking $3.3 \cdot 10^8$ T_{act} CBA-anti-(CBA × C57BL/6)F_1 or T_{act} CBA-anti-BALB/C, or $4.5 \cdot 10^8$ spleen, thymus, and lymph node cells of (CBA × C57BL/6)F_1 mice, in equal proportions, to 1 ml of antiserum.

Laboratory of Immunologic Tolerance, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. Ya. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 6, pp. 708-710, June, 1980. Original article submitted June 6, 1979.

TABLE 1. Activity of Different Batches of $AT_{act}S$ in the Cytotoxic Test

Batch No. of $AT_{act}S$	Maximal CTI* (in %) when testing $AT_{act}S$ on different target cells								
		T_{act}							
	NLy†	CBA-anti-(CBA × C57BL/6)F_1 H-2^k, Ala-1·1	CBA-anti-BALB/C H-2^k, Ala-1·1	CBA-anti-DBA/2 H-2^k, Ala-1·1	CBA-anti-(CBA × C3H-OH)F_1 H-2^k, Ala-1·1	C3H-anti-C57BL/6 H-2^k, Ala-1·1	BALB/C-anti (CBA×C57BL/6)F_1 H-2^d, Ala-1·1	C57BL/6-anti BALB/c H-2^b, Ala-1·2	C57BL/6-anti (CBA×C57BL/6)F_1 H-2^b, Ala-1·2
I	4	68	46	45	56	—	49	—	—
II	3	43	33	—	—	44	33	—	—
III	4	53	42	—	—	—	—	38	—
IV	1	49	39	—	—	—	—	—	31
V	5	49	25	—	—	—	—	—	—

*CTI cytotoxic index with dilutions of $AT_{act}S$ from 1:4 to 1:12.
†NLy) thymus, lymph node, and spleen cells of intact CBA, BALB/C, and (CBA × C57BL/6)F_1 mice.

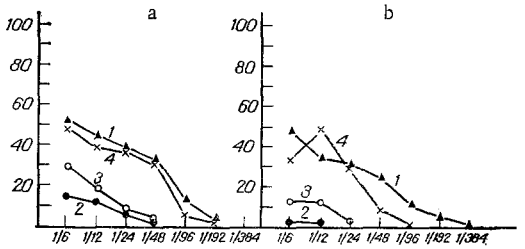

Fig. 1. Inhibition of cytotoxic activity by $AT_{act}S$ after absorption by intact and activated lymphocytes (combined data for batches I, II, and IV of $AT_{act}S$). CBA-anti-(CBA × C57BL/6)F_1 T_{act} (a) and CBA-anti-BALB/C T_{act} (b) were used as the target cells. $AT_{act}S$: 1) before absorption; 2) after absorption by CBA-anti-(CBA × C57BL/6)F_1 T_{act}; 3) after absorption by CBA-anti-BALB/C T_{act}; 4) after absorption by intact lymphocytes. Ordinate, cytotoxic index (in %); abscissa, dilution of antiserum.

EXPERIMENTAL RESULTS

It was shown as a first step that living cells obtained from spleens of irradiated recipients 3 days after injection of the donor's thymocytes were T cells of the donor's phenotype: In the cytotoxic test all the cells died following exposure to ATS and antilinear serum which reacted with intact donor's, but not recipients', cells.

Data showing the action of the five batches of $AT_{act}S$ on different target cells are given in Table 1. Clearly, each $AT_{act}S$ had virtually no effect on the thymus, lymph node, and spleen cells of intact mice (cytotoxic index 1-5%). Meanwhile, all batches of $AT_{act}S$ were active against T_{act}: they killed 25-68% of cells in populations of T lymphocytes activated by different allogeneic transplantation antigens. The highest activity of each batch of $AT_{act}S$ was directed against CBA-anti-(CBA × C57BL/6)F_1 target cells, which were used as immunizing material for obtaining the $AT_{act}S$. Special experiments (the results are not given) showed that the selective cytotoxic action of $AT_{act}S$ on T_{act} was not associated with their greater sensitivity to treatment with antisera in general: ATS and ALS had the same cytotoxic action on T_{act} and intact lymphocytes. On the basis of these results the presence of a specific differential antigen on the surface of T_{act} can be postulated. Data on the ability of T_{act} and intact lymphocytes (Fig. 1) to absorb $AT_{act}S$ confirm this suggestion. Absorp-

tion on $AT_{act}S$ by CBA-anti-(CBA × C57BL/6)F_1 and CBA-anti-BALB/C T_{act} reduced the cytotoxic activity of the $AT_{act}S$ by a much greater degree than absorption by intact lymphocytes. For example, for CBA-anti-(CBA × C57BL/6)F_1 target cells (Fig. 1a) in the first two dilutions activity of $AT_{act}S$ was reduced by 46-72% after absorption by T_{act}, whereas after absorption by intact lymphocytes it was reduced by only 10%. A similar result was obtained for CBA-anti-BALB/C target cells (Fig. 1b). In the first two dilutions before absorption the $AT_{act}S$ killed 47 and 34% of target cells. $AT_{act}S$ absorbed by CBA-anti-(CBA × C57BL/6)F_1 T_{act} completely ceased to react with CBA-anti-BALB/C cells, but after absorption by CBA-anti-BALB/C T_{act} it killed only 12-13% of the target cells. In these same dilutions, $AT_{act}S$ absorbed by intact lymphocytes killed 33 and 48% of CBA-anti-BALB/C cells.

It can be concluded from these findings that a specific differential antigen can be detected on mouse T cells activated by allogeneic transplantation antigens, and also that antibodies can be obtained against this antigen which possess selective cytotoxicity relative to T_{act} and do not affect intact lymphocytes.

Our results agree with those obtained by Feeney and Hämmerling [3, 4], who detected a specific antigen of activated Ala-1 lymphocytes by the use of allogeneic antiserum against mouse lymphocytes transformed by phytohemagglutinin. However, by contrast with the data of these workers, the antiserum we obtained reacted equally with activated cells of different H-2 and Ala-1 phenotype (Table 1). Hence, it can be suggested that the antigen we discovered is not identical with the Ala-1 antigen. According to data in the literature [8], and also obtained with the aid of xenogeneic antibodies, a differential antigen of activated T cells (T killers) has been found. Further investigations in this direction will evidently enable the production of a special type of immunosuppression, associated with elimination of only activated lymphocytes by antibodies.

LITERATURE CITED

1. V. G. Nesterenko and L. V. Koval'chuk, Byull. Éksp. Biol. Med., No. 7, 836 (1976).
2. L. N. Fontalin, L. A. Pevnitskii, and V. V. Solov'ev, Vestn. Akad. Med. Nauk SSSR, No. 2, 78 (1967).
3. A. J. Feeney and U. Hämmerling, Immunogenetics, 3, 369 (1976).
4. A. J. Feeney and U. Hämmerling, J. Immunol., 118, 1488 (1977).
5. I. H. Helderman and T. B. Strom, Nature, 274, 62 (1978).
6. P. J. Robinson and B. H. Anderton, Exp. Cell Res., 102, 431 (1976).
7. J. Sprent and J. F. A. P. Miller, Cell. Immunol., 3, 213 (1972).
8. R. A. Sullivan, G. Berke, and D. B. Amos, Transplantation, 16, 388 (1973).