DISCOVERY OF A SPECIAL DIFFERENTIAL ANTIGEN (Aca-1) OF ACTIVATED MOUSE T- AND B-LYMPHOCYTES

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With the aid of specific heterologous antisera the writers previously showed the presence of a special antigen on mouse T-lymphocytes stimulated by phytohemagglutinin [1] or by allogeneic transplantation antigens [2, 3]. In the present investigation an attempt was made to discover a common differential antigen of activated T- and B-lymphocytes. It was suggested that such an antigen might be found on activated mouse B-lymphocytes by the use of specific heterologous antibodies against mouse T-cells activated by transplantation antigens (T_{act}).

EXPERIMENTAL METHOD

Experiments were carried out on male CBA ($H\text{-}2^k$), C57BL/6 ($H\text{-}2^b$), DBA/2($H\text{-}2^d$), BALB/c ($H\text{-}2^d$), C3H($H\text{-}2^k$), AKR($H\text{-}2^k$), and CC57BR ($H\text{-}2^b$) mice. An EL-4 T-lymphoma, transplanted to C57BL/10Sn ($H\text{-}2^b$) mice, also was used. Immunoglobin (Ig) and IgM were isolated from sera of CBA, C57BL/6, and BALB/c mice with transplanted MOPC-21 and MOPC-104E plasmacytomas; antisera against mouse IgG were obtained from rabbits.

Antiserum against T-lymphocytes activated by transplantation antigens ($AT_{act}S$) was obtained by the method described previously [2]: 10^8 thymocytes of CBA mice were injected intravenously into (CBA × C57BL/6)F_1 hybrids, irradiated in a dose of 850 R. After 3 days cell suspensions containing transformed lymphocytes were prepared from the recipients' spleens [7]. Rabbits were immunized with these suspensions. The immune rabbit serum was absorbed by liver, erythrocytes, serum, and also thymus, spleen, and lymph node cells of intact CBA, C57BL/6, and (CBA × C57BL/6)F_1 mice. Absorption was continued until all activity of the sera had disappeared in the hemagglutination test with mouse erythrocytes, in the double diffusion test in gel with serum and Ig of intact mice, and in the cytotoxic test with intact lymphocytes [1]. Five batches of $AT_{act}S$ were obtained and all acted similarly.

The effect of $AT_{act}S$ on activated B-lymphocytes was estimated by its action on antibody-forming cells (AFC), producing IgM antibodies against erythrocytes of sheep, August rats, and rabbits, and also on AFC producing IgG antibodies against sheep's erythrocytes. For this purpose spleen cells of mice immunized intravenously 4-13 days before the experiments with 5×10^8 erythrocytes were treated *in vitro* with antiserum (in dilutions of 1:50-1:1250) with rabbit complement and the number of direct and indirect AFC was determined by Jerne's method [4]. The inhibitory action of the antisera was assessed by calculating the percentage inhibition of AFC, equal to $(a - b)/a \cdot 100\%$, where a is the number of AFC when the cells were treated with complement only, b the number of AFC when treated with antiserum + complement.

Special additional absorption of $AT_{act}S$ was carried out by activated T-lymphocytes [T_{act} CBA-anti-(CBA × C57BL/6)F_1 or T_{act} CBA-anti-BALB/c] or with EL-4 T-lymphoma cells. The serum was absorbed for 1 h at room temperature with $3.3 \cdot 10^8$ cells to 1 ml serum. In the control experiments the serum was absorbed by $4.5 \cdot 10^8$ spleen, thymus, and lymph node cells, taken in equal proportions, from intact (CBA × C57BL/6)F_1 mice.

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TABLE 1. Inhibitory Effect of $AT_{act}S$ on AFC Producing Antibodies against Sheep's, Rat's, and Rabbit's Erythrocytes

Dilution of $AT_{act}S$	Reduction (in %) in number of AFC* of different strains of mice producing antibodies against:									
	sheep's erythrocytes								rat's erythrocytes†	rabbit's erythrocytes†
	(CBA × C57BL/6) F_1 H-2k/b Ala-1,1/1,2	CBA H-2^k Ala-1,1	BALB/c H-2^d Ala-1,1	A/Sn H-2^a Ala-1,1	AKR H-2^k Ala-1,2	C57BL/6 H-2^k Ala-1,2	DBA/2 H-2^d Ala-1,2	CC57BR H-2^b		
1:50	98	99	91	95	96	100	98	99	100	100
1:250	94	72	46	36	84	62	76	73		
1:1250	24	0	0	13	19	0	0	0		

*AFC determined in mouse spleens four days after intravenous immunization with $5 \cdot 10^8$ erythrocytes.
†AFC detected in spleens of (CBA × C57BL/6)F_1 mice.

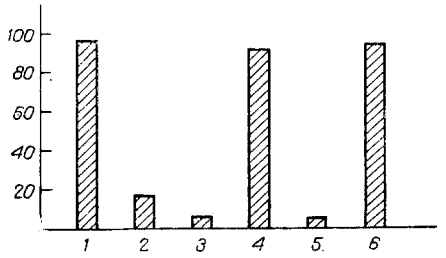

Fig. 1

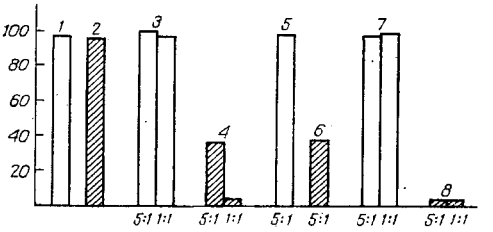

Fig. 2

Fig. 1. Reduction of ability of $AT_{act}S$ to inhibit AFC of (CBA × C57BL/6)F_1 mice after absorption of serum by intact lymphocytes, T_{act}, and EL-4 T-lymphoma cells. Abscissa: I) $AT_{act}S$ before absorption, $AT_{act}S$ after absorption by cells; 2) CBA-anti-(CBA × C57BL/6)F_1 T_{act}; 3) CBA-anti-BALB/c T_{act}; 4) intact lymphocytes; 5) EL-4 cells; 6) liver cells; ordinate: inhibition of AFC, in %. Dilution of $AT_{act}S$ 1/250.

Fig. 2. Effect of Ig, IgM, and whole serum of intact mice on activity of $AT_{act}S$. Abscissa: 1) $AT_{act}S$; 2) AIgS; 3) $AT_{act}S$ + Ig; 4) AIgS + Ig; 5) $AT_{act}S$ + IgM; 6) AIgS + IgM; 7) $AT_{act}S$ + mouse serum; 8) AIgS + mouse serum; ordinate, percent inhibition of AFC. Unshaded columns denote $AT_{act}S$, obliquely shaded columns AIgS. Ratios between $AT_{act}S$ and neutralizing material by volume shown below columns.

To study the inhibitory action of Ig, IgM, or intact mouse serum on the cytotoxic activity of $AT_{act}S$ the following technique was used: Before addition to AFC the $AT_{act}S$ was incubated for 10 min at room temperature with total Ig, IgM, or intact mouse serum, after which the AFC were treated with this mixture in the presence of rabbit complement.

EXPERIMENTAL RESULTS

Data on the effect of $AT_{act}S$ on AFC producing IgM antibodies against sheep, rat, and rabbit erythrocytes are given in Table 1. They show that $AT_{act}S$ inhibited AFC producing antibodies against all three antigens. In a dilution of 1:50, for instance, it inhibited AFC by 91-100%. The action of $AC_{act}S$ was not strain-specific: $AT_{act}S$ inhibited AFC of different H-2 and Ala-1 phenotypes. Experiments to show the effect of $AT_{act}S$ on indirect AFC (the results are not given) also showed that the experimental serum, in a dilution of 1:50, inhibited by 95-100% indirect AFC from CBA (H-2^k, Ala-1, 1), C57BL6-(H2^b, Ala-1, 2), (CBA × C57BL/6)F_1 (H-$2^{k/b}$, Ala-1,1/1,2) mice obtained 7-13 days after immunization with sheep's erythrocytes. Without complement, the $AT_{act}S$ did not act on the AFC.

These results suggested the presence of a common differential antigen on the surface of the activated T- and B-lymphocytes. The results which showed that T_{act}, EL-4 T-lymphoma cells, and intact lymphocytes can absorb $AT_{act}S$ confirm this hypothesis (Fig. 1). Absorption of the antiserum by activated CBA-anti-(CBA × C57BL/6)F_1 and CBA-anti-BALB/c T-lymphocytes (T_{act}) and by EL-4 T-lymphoma cells inhibited the cytotoxic activity of the $AT_{act}S$ by 83-95%.

Meanwhile, absorption with intact lymphocytes or liver cells of intact mice caused virtually no change in its activity.

An attempt also was made to inhibit activity of $AT_{act}S$ with Ig, IgM, and whole serum of intact mice (Fig. 2). The results of these experiments showed that $AT_{act}S$ and antiserum against mouse IgG (AIgS) inhibited AFC by 96 and 95%, respectively. Preliminary incubation of the $AT_{act}S$ with total Ig, IgM, or whole serum of intact mice did not reduce its inhibitory activity. Meanwhile, similar incubation of AIgS with total Ig, IgM, or whole serum of intact mice reduced its inhibitory action by 60-100%. These data, as well as others indicating insensitivity of lymph node and spleen cells of intact mice (which included 30-40% of B-lymphocytes with Ig on the surface) in the cytotoxic test to the action of $AT_{act}S$, and a decrease in activity of $AT_{act}S$ after absorption by EL-4 T-lymphoma cells (on the surface of which no Ig could be detected), and the absence of reaction in the doublediffusion test in gel between $AT_{act}S$, on the one hand, and total Ig and intact mouse serum, on the other, suggest that the cytotoxic activity in $AT_{act}S$ is not connected with anti-immunoglobulin antibodies.

Previously a special differential antigen of activated T-lymphocytes was demonstrated with the aid of $AT_{act}S$ [2, 3]. It was found on T_{act} of different H-2 and Ala-1 phenotypes and on the surface of some EL-4 T-lymphoma cells, but could not be found on intact lymphocytes. The data given in this paper indicate the presence of a similar antigen also on activated B-lymphocytes (AFC). $AT_{act}S$ inhibited direct and indirect AFC of different H-2 and Ala-1 phenotypes and its cytotoxic activity was specifically reduced after absorption with activated T-cells but not with intact lymphocytes. The discovery of a special antigen of activated T- and B-cells, namely Ala-1, with the aid of allogeneic antiserum against phytohemagglutinin-transformed lymphocytes, has been described in the literature [5, 6]. However, by contrast with the results published by these workers, the antiserum we obtained reacted equally with activated T-cells [2, 3] and B-cells (AFC) of different H-2 and Ala-1 phenotypes. This suggests that the antigen we discovered is not identical with Ala-1 antigen. We propose calling it Aca-1 (activated cells antigen). In our opinion heterologous antibodies against Aca-1 may prove useful both for studying cell differentiation and during the development of new immunodepressive agents, reacting only with activated cells.

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