EFFECT OF NORMAL MOUSE SERA AND ALLOANTIBODIES AGAINST LYMPHOCYTES AND TARGET CELLS ON CYTOTOXIC ACTIVITY OF IMMUNE LYMPHOCYTES

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The cytotoxic effect of immune lymphocytes on allogeneic target cells was depressed in proportion to the concentration of normal mouse serum in the culture medium. The intensity of depression of the cytotoxic effect of the alloantiserum against antigens of the target cells was the same as that of normal serum. Alloantibodies against the donor line of lymphocytes, on the other hand, intensified the cytotoxic effect while preserving its immunologic specificity. This action of the antibodies was abolished by absorption of the alloantiserum with the corresponding lymphocytes.

Interaction between humoral alloantibodies and immune lymphocytes is regarded as one of the factors determining the fate of a graft. By blocking transplantation antigens, alloantibodies promote or prevent the cytotoxic effect of the lymphocytes depending on the concrete conditions [1, 5, 15]. Studies of this problem on an in vitro model of cellular immunity have shown that treatment of target cells with the corresponding alloantibodies did not prevent the cytotoxic effect of the immune lymphocytes if the antibodies were removed before addition of the lymphocytes [3]. Conversely, the activity of the immune lymphocytes was suppressed if they were added in the presence of an excess of antiserum against target cells [7, 13]. Antibodies reacting only with a small proportion of antigens of the target cells against which the lymphocytes exerted their action did not depress the cytotoxic effect [8, 12]. A study of antitumor immunity has shown that after absorption or removal of tumors from mice , rabbits, and man their sera did not depress the cytotoxic action of syngeneic lymphocytes in vitro on the tumor cells, but they did possess this property if they were obtained from donors with a progressively developing tumor [10].

In all these cases, the syngeneic or allogeneic antibodies were directed against target cells. The action of antilymphocytic alloantibodies on the activity of immune lymphocytes has not been studied.

The object of the present investigation was to study the effect of normal and immune mouse sera against antigens of lymphocytes or target cells on the activity of immune lymphocytes.

EXPERIMENTAL METHOD

Mice of inbred lines C57BL/10Sn (H-2^b), CC57BR (H-2^b), and B10 · D2 (H-2^d) were obtained from the nursery of the N. F. Gamaleya Institute of Epidemiology and Microbiology and used at the age of 8-16 weeks. The tumors used were sarcomas induced by methylcholanthrene and reinoculated into C57BL/10Sn and B10.D2 mice. C57BL anti-B10.D2 and D10.D2 anti-C57BL antisera were obtained by immunization with 6 doses of allogeneic cells [2] and were kept at −20°C. The antibodies were determined by the dextran hemagglutination test [9]. The titer of the sera varied from 9 to 12 $\log_2$.

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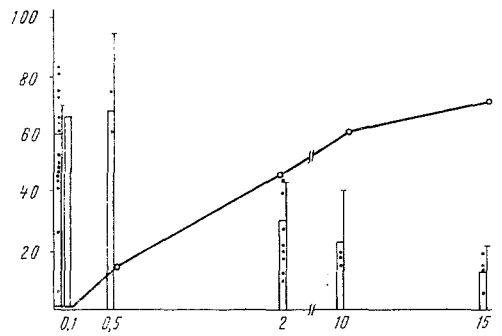

Fig. 1. Effect of normal mouse serum on CE of immune lymphocytes. Abscissa, final concentration of serum in medium (in %); ordinate, CE (in %; columns) and depression of CE (in %; curve). Points denote results of individual experiments, vertical lines show confidence intervals for a 95% level of significance.

The target cells consisted of 2-day primary monolayer cultures of peritoneal macrophages obtained with the aid of an irritant and seeded in a concentration of 2×10^5/ml in flat-sided test tubes.

The experiments to study the cytotoxic effect (CE) were carried out by the method described previously [3, 4]. Immune and normal lymphocytes from mice of the same line were washed 3 times and the number of living cells counted, after which they were suspended in medium No. 199 and added in equal doses in a volume of 1 ml to washed cultures of target cells. The dose of lymphocytes varied from 5×10^6 to 20×10^6 per tube, and each sample of lymphocytes was added to 4 tubes. The medium was replaced after 24 h by medium No. 199 with 2% bovine serum, and the number of living macrophages was counted next day, and the value of CE determined. Statistical significance was assessed by Student's t-test.

To treat the target cells with the sera, washed cultures were incubated for 1 h at 37°C in a volume of 0.5 ml, after which an equal volume of suspension of lymphocytes was added. When the lymphocytes were treated with the sera, from 20×10^6 to 80×10^6 of sedimented cells were suspended in 0.8 ml of different dilutions of sera or of medium No. 199, and incubated for 1 h at 37°C, after which 3.2 ml medium No. 199 was added to each sample and the mixture added to the cultures in doses of 1 ml.

Complete exhaustion of the antibodies by allogeneic spleen cells was carried out by incubation of 37°C for 1 h in the proportion of 10^8 cells to 1 ml serum diluted 1:10. Completeness of exhaustion was verified by dextran hemagglutination.

EXPERIMENTAL RESULTS

The CE of the immune lymphocytes in the presence of normal mouse serum was studied in 16 experiments. As Fig. 1 shows, the CE, the mean value of which was 59%, was reduced by 15, 47, 61, and 71% after the addition of 0.5, 2, 10, and 15% normal serum respectively to the medium. This depression of CE is highly significant ($P < 0.01$) in the presence of serum in concentrations of 2% or more.

Depression of CE by normal serum and by antiserum against target cells was the subject of parallel tests in 7 experiments. The final dilution of the sera in the medium varied from 1 to 20% and the final titer of antibodies from 4 to 9 $\log_2$. The CE of the C57BL anti-B10.D2 lymphocytes was usually 1.5 times less than the CE of the B10.D2 anti-C57BL lymphocytes for equal doses.

The results in Fig. 2 show that the CE of C57BL anti-B10.D2 lymphocytes was reduced by 25-100% in proportion to the increase in concentration of normal serum. The CE of the B10.D2 anti-C57BL lymphocytes was not significantly changed by serum in a concentration of 2%, but was reduced on the average by 55% by serum in a concentration of 20%. In no case was the depression of CE in the presence of antiserum against target cell antigens significantly different from that in the presence of normal serum.

The effect of antilymphocytic B10.D2 anti-C57BL antibodies on CE of C57BL anti-B10.D2 lymphocytes was studied in 9 experiments. The mean value of CE of the untreated immune lymphocytes was 62% (Fig. 3). As in the previous experiments, CE was reduced by the addition of normal serum to the medium, but it increased again if antilymphocytic antiserum was used. As Fig. 3 shows, the increase in CE due to the action of antiserum, compared with normal serum, averaged 36, 75, and 135% for final concentrations of sera in the medium of 0.4, 2, and 10% respectively, and in the last 2 cases it was highly significant ($P < 0.01$) in all experiments.

An increase in CE was discovered only if antilymphocytic antibodies were constantly present in the medium, because the activity of lymphocytes washed to remove serum after preliminary treatment was indistinguishable from the CE of the original immune lymphocytes.

The presence of antilymphocytic alloantibodies in the medium did not change the specificity of the CE: C57BL anti-D10.D2 lymphocytes had no CE on syngeneic C57BL macrophases, and normal lymphocytes did not become active (Fig. 3).

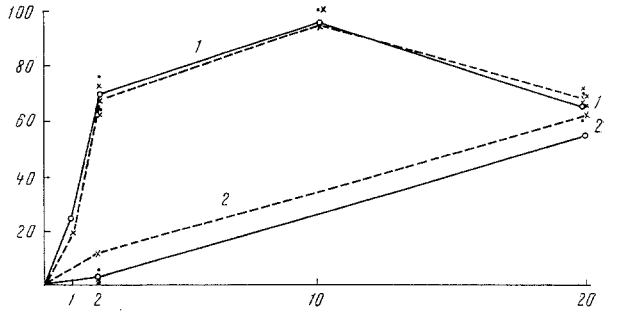

Fig. 2. Effect of antibodies against target cells on CE of immune lymphocytes. Abscissa, final concentration of serum in medium (in %); ordinate, depression of CE (in %). Continuous lines represent normal sera, broken lines antisera. 1) Lymphocytes and C57BL anti-B10.D2 serum; 2) B10.D2 anti-C57BL serum.

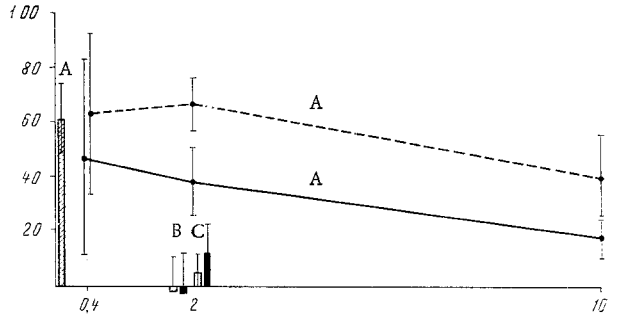

Fig. 3. Effect of B10.D2 anti-C57BL antibodies on CE of C57BL anti-B10.D2 lymphocytes. Abscissa, final concentration of serum in medium (in %); ordinate, CE (in %). Continuous line and unshaded columns represent normal serum; broken line and black columns, antiserum; shaded columns, no serum. A) Action of immune lymphocytes of allogeneic B10.D2 target cells; B) action of immune lymphocytes on syngeneic C57BL target cells; C) action of normal C57BL lymphocytes on allogeneic B10.D2 target cells. Vertical lines denote confidence intervals for a 95% level of significance.

To demonstrate the causal role of antilymphocytic antibodies in the increase in CE, B10.D2 anti-C57BL serum was absorbed with CC57BR lymphocytes possessing antigens of the same H-2 allele as C57BL. As Table 1 shows, the increase in CE in the presence of absorbed serum was much lower than the increase in CE in the presence of the original serum. Control absorption by syngeneic B10.D2 lymphocytes did not reduce the increase in CE.

The results indicate that depression of the CE of immune lymphocytes by antibodies against target cells is not specific: the analogous result was observed in the presence of normal mouse serum. These findings do not agree with those cited above [7, 12, 13]. The discrepancy could be due to differences in the experimental conditions: in the work of Brunner et al. [7], a suspension culture was used as the target cells, and the CE was estimated from the elimination of Cr^{51} from the cells or inhibition of growth of the colonies. In 2 of the 3 experiments described by Möller [13], the CE was also depressed in the presence of normal serum, although to a lesser degree than by antiserum. In the present experiments, depression of the CE by normal mouse serum was associated with depression of the adsorption of immune lymphocytes on the target cells. The serum of other species (bovine, for example) did not possess this action. Judging from the preliminary data, the active principle in mouse serum is α-globulin.

TABLE 1. Absorption of Alloantibodies Stimulating Cytotoxic Effect (in %) of C57BL Anti-B10.D2 Lymphocytes

None added	Added to medium No. 199 serum B10.D2 15%-3%*					
	normal	unabsorbed	anti-C57BL antiserum absorbed by cells			
			CC57BR (expt.)	P†	B10.D2 (control)	P†
1	2	3	4	5		
82,6	67,5	80,7 (19)	61,7 (0)	<0,05	83,6 (24)	>0,1
71,7	40,0	66,3 (66)	50,2 (25)	<0,01	64,3 (61)	>0,1
81,1	34,4	60,5 (76)	—	—	77,5 (125)	>0,05
46,7	9,8	40,8 (316)	18,0 (84)	<0,05	—	—

*First number denotes concentration during preliminary treatment of lymphocytes, second number final concentration in medium.
†Differences from results in column 3.
Note. Increase in cytotoxic effect in parentheses (in percent relative to values in column 2).

The mechanisms of destruction of target cells have been the subject of lively debate in recent years. One suggestion is that this destruction is due to "allogeneic inhibition," i.e., to close contact between antigenically unidentical surfaces of lymphocytes and target cells. The role of "immunity" of the lymphocytes is merely to facilitate this contact [11]. If this hypothesis is correct, antibodies against lymphocytes, by blocking their antigens, must prevent contact with foreign antigens and depress CE, as was observed when normal mouse lymphocytes, stimulated by phytohemagglutinin, were used [14].

The results of the present investigation do not support this hypothesis: antilymphocytic alloantibodies not only did not depress but, on the contrary, they stimulated the CE of the immune lymphocytes compared with that in the presence of normal serum. Similar results [12] were published after the completion of this investigation. Destruction of target cells by immune and "activated" normal lymphocytes is evidently due to different mechanisms.

The increase in CE by the action of antilymphocytic antibodies may be associated with a decrease in the charge on the surface of the lymphocytes on contact with antibodies [6, 16] and with a subsequent increase in their adsorption on target cells or with activation of intracellular processes essential to CE in the lymphocytes. This problem requires further study.

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