The antigens discovered are interesting because they may be antigenic markers for malignant transformation of certain types of cells: Their absence or presence in human malignant tumors must evidently correlate with whether the tumor arises from the cell producing them or from a cell not producing them.

The discovery of these antigens in stable human cell lines deserves attention: All HCL synthesizing them have for a long time been maintained as an infinitely transplantable line, and as a result of prolonged culture they must have lost their differentiated cells. Since the stomach is a derivative of the entoderm, and the breast and larynx (HeP-2) are derivatives of the ectoderm, it is difficult to postulate the participation of a common stem cell in the synthesis of the common antigens.

LITERATURE CITED

1. A. I. Gusev and A. K. Yazova, Byull. Éksp. Biol. Med., No. 4, 120 (1970).
2. K. V. Il'in, A. F. Bykovskii, and Zh. Zh. Spure, Byull. Éksp. Biol. Med., No. 2, 86 (1972).
3. D. A. Él'gort and G. I. Abelev, Byull. Éksp. Biol. Med., No. 2, 118 (1971).
4. D. Colcher, S. Spiegelman, and J. Schlom, Proc. Nat. Acad. Sci. USA, 71, 4975 (1974).
5. P. Gold and S. O. Freedman, J. Exp. Med., 121, 439 (1965).
6. K. V. Ilyin (K. V. Il'in), I. S. Irlin, and A. P. Bykovsky (A. F. Bykovskii), Cancer (Philadelphia), 34, 532 (1974).
7. I. N. Kryukova, K. V. Ilyin (K. V. Il'in), N. N. Mazurenko, et al., Neoplasma (Bratislava), 15, 235 (1975).
8. R. Reisfeld and M. Pellegrino, Science, 172, 1134 (1971).
9. G. Seman, S. Hunter, R. Miller, et al., Cancer (Philadelphia), 37, 1814 (1976).
10. J. Yeh, M. Ahmed, S. A. Mayyasi, et al., Science, 190, 583 (1975).

ANTIBODY FORMATION AGAINST ANTIGEN-RECOGNIZING RECEPTORS OF T LYMPHOCYTES IN A SYNGENEIC SYSTEM

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Antibody formation against antigen-recognizing receptors of T lymphocytes were shown to be capable of being formed in a syngeneic system. Antiserum of CBA mice receiving intravenous injections of CBA lymphocytes immune against C57BL cells specifically inhibited blast transformation of CBA T lymphocytes against C57BL cells only in a mixed culture. The same antiserum had no effect on proliferative activity of CBA T lymphocytes reacting to "foreign antigen" – i.e., DBA/2 cells. No antibodies against C57BL cells likewise were found in the antireceptor antiserum. A regulatory influence of autoantireceptor antibodies on the immune response is postulated.

KEY WORDS: antigen-recognizing receptor; antireceptor serum; blast transformation

In recent investigations [2, 3, 7, 8, 13, 14, 16] so-called antireceptor sera, which specifically inhibit the response of lymphocytes to one antigen only without affecting immunoreactivity to other antigens, have been obtained in a xenogeneic or semiallogeneic system. It has been suggested [9, 12, 15, 17, 18] that the production of autoantireceptor antibodies, which may play an essential role in the regulation of the immune response, can take place in the body in situ.

The object of the present investigation was to study the possibility of formation of antireceptor antibodies in a syngeneic system and to examine their effect on T-lymphocyte function. The aim was to obtain antibodies specifically inhibiting proliferation of T lymphocytes of mice of one strain only against cells of mice of another strain in a syngeneic system in a unidirectional mixed lymphocyte culture.

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TABLE 1. Effect of Antireceptor Serum on Antigen-Recognizing Cells

Group	Responding cells	Stimulating cells	Number of counts/min	Coefficient of stimulation
1	CBA+ ARS	C57BL	2425±895	0,8
2	CBA+ ARS	DBA/2	34 680±11 989	11,2
3	C57BL+ ARS	CBA	21 543±7035	7,4
4	CBA+ normal serum	C57BL	32 820±12 164	10,6
5	CBA+ "	DBA/2	26 502±8733	8,5
6	C57BL+ "	CBA	27 474±6129	9,5
7	CBA+ "	CBA	3109±1541	1,0
8	C57BL+ "	C57BL	2897±863	1,0

EXPERIMENTAL METHOD

Male mice of strains CBA/H (H-2^k, Mls^b), C57BL/6 (H-2^b, Mls^b), and DBA/2 (H-2^d, Mls^a), obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, and weighing 18-20 g were used.

Antireceptor antiserum (ARS) was obtained by immunizing intact CBA mice intravenously, twice at an interval of 2 weeks, with regional lymph node cells of CBA mice (10^8 cells per immunization), which had been given subcutaneous injections of 10^8 thymus cells of C57BL mice at 6 points 7 days before sacrifice. The mice were exsanguinated 10 days after the second immunization. A mixture of the blood sera of 15 mice was used in the experiments.

For lymphocyte culture in vitro, mouse spleen cells were kept for 4 days in silicone-treated penicillin flasks ($5 \cdot 10^6$/ml) in 2 ml of culture medium of the following composition: 5% calf embryonic serum, 1% L-glutamine, $5 \cdot 10^{-3}$ M HEPES, and $3 \cdot 10^{-5}$ M 2-mercaptoethanol were added to RPMI-1640 medium. [^{3}H]Thymidine (4 μCi, specific activity 1 Ci/mmole) was added to the medium 4 h before the end of culture. Proliferative acitivity of the cell suspensions was assessed by a radiometric method based on the incorporation of [^{3}H]-thymidine into DNA of the proliferating cells, by means of a "Packard" scintillation counter in the usual way [4]. To assess the intensity of the blast transformation reaction the coefficient of stimulation was calculated by the formula a/b, where a is the number of counts in the experimental culture and b the number of counts in the control syngeneic cultures. Intact mouse spleen cells were used as the responding cells, and similar cells irradiated in a dose of 1500 R (^{60}Co γ rays, ÉKU-50 apparatus) as the stimulating cells. The ratio between responding and stimulating cells in the cultures was 3 : 7.

To assess the effect of the antisera on antigen-recognizing lymphocytes, before addition to the culture the responding cells were treated in vitro with antisera in the presence of complement [5], after which the twice washed cells were added to the stimulating cells. All manipulations were carried out under sterile conditions in the cold.

Activity of the antisera also was tested in the lymphocytotoxic test by determining the viability of the cells with the aid of trypan blue [4].

Antilinear CBE anti-C57BL antiserum obtained by the usual method [5] and rabbit antiserum against mouse T lymphocytes also were used. The globulin isolated from this rabbit antiserum (ATG) was kindly provided by the Laboratory of Immunology of the Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR [1].

The specificity and high activity of the antilinear CBA anti-CBL serum and of the ATG were tested in the cytotoxic test and by their effect on cells producing antibodies against sheep's red cells [6]. In these tests the antilinear serum acted only against cells of C57BL mice and did not react with cells of CBA mice. ATG in the cytotoxic test caused death of 100% of thymus cells but did not affect bone marrow cells or antibody-forming cells. The cytotoxic test of the antilinear CBA anti-C57BL serum and of the ATG with thymus cells was 1 : 512 and 1 : 320 respectively.

EXPERIMENTAL RESULTS

It was first shown that, in agreement with data in the literature [10, 11], the responding cells in a mixed lymphocyte culture were T lymphocytes: Treatment of the responding cells with ATG inhibited blast transformation by 96%.

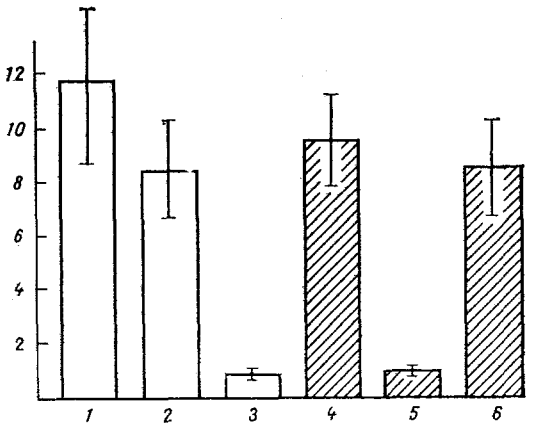

Fig. 1. Effect of antireceptor serum and antilinear CBA anti-C57BL serum on antigen-recognizing CBA and C57BL cells in a mixed lymphocyte culture. 1-3) CBA cells treated with antisera and stimulated by irradiated C57BL cells; 4-6) C57BL cells treated with antisera and stimulated by irradiated CBA cells; 1, 4) normal serum; 2, 5) CBA anti-C57BL serum; 3, 6) antireceptor antiserum. Ordinate) coefficient of stimulation.

Data on the effect of ARS on the responding cells are given in Table 1. ARS inhibited proliferation of CBA lymphocytes responding to "homologous" antigen, i.e., cells of C57BL mice in group 1, by 93%. Meanwhile it caused virtually no inhibition of blast transformation of CBA lymphocytes to "foreign" antigen, namely cells of DBA/2 mice (group 2). The ARS thus contained antibodies against CBA lymphocytes responding only with cells of C57BL mice, and contained no antibodies against CBA lymphocytes of other specificities. No antibodies against lymphocytes of C57BL mice likewise were found in ARS: It did not suppress the response of the lymphocytes of C57BL mice against cells of CBA mice in the mixed culture (group 3).

In the cytotoxic test, ARS did not cause visible death of the cells of the thymus, spleen, lymph nodes, and bone marrow of CBA and C57BL mice, i.e., the ARS contained no antibodies against the main mass of lymphocytes and evidently attacked only a very small proportion of the T cells.

It might be suggested that the test serum (ARS) contained blocking antibodies reacting with the stimulating cells, which would explain its specific inhibitor effect. To test this hypothesis the ARS was compared with antiserum known to contain antibodies against C57BL cells, namely antilinear CBA anti-C57BL serum. The results are given in Fig. 1. The ARS and antilinear CBA anti-C57BL serum possessed directly opposite actions on the responding CBA and C57BL cells. ARS abolished blast transformation of CBA lymphocytes stimulated by C57BL cells but did not affect the response of the C57BL lymphocytes to CBA cells. Conversely, as was expected, the antilinear serum specifically inhibited the proliferation of C57BL lymphocytes during culture with CBA cells and virtually did not suppress the response of CBA lymphocytes to C57BL cells. These results confirm once again that the object of the specific action of the antisera in this experimental model was the antigen-recognizing responding lymphocytes.

It can be concluded from these results that an antireceptor antiserum specifically inhibiting proliferation of the T lymphocytes of CBA mice only against cells of C57BL mice was obtained in a syngeneic system. Presumably antibodies against the antigen-recognizing receptors of the T lymphocytes are also formed in situ (in an autologous system), when they play an important role in the regulation of the immune response (for example, bringing it to an end).

The use of antireceptor antibodies may perhaps provide a new approach to the study of the problem of the structure of the antigen-recognizing receptors of lymphocytes. In the writers' opinion, the use of antireceptor antibodies may also help to obtain highly specific clonal immunosuppression.

LITERATURE CITED

1. N. A. Kraskina, in: Proceedings of the All-Union Conference on General and Applied Immunology [in Russian], Part 1, Moscow (1974), pp. 78-79.
2. N. A. Kraskina, Trudy Moskovsk. Nauch.-Issled. Inst. Épidemiol. Mikrobiol., 17, 171 (1976).
3. V. G. Nesterenko, Trudy Moskovsk. Nauch.-Issled. Inst. Épidemiol. Mikrobiol., 17, 187 (1976).

4. V. G. Nesterenko, "Immunological properties and antigenic structure of a population of lymphocytes stimulated by a mitogen or antigen," Author's Abstract of Candidate's Dissertation, Moscow (1977).
5. V. G. Nesterenko and L. V. Koval'chuk, Byull. Éksp. Biol. Med., No. 9, 1096 (1976).
6. L. N. Fontalin, L. A. Pevnitskii, and V. V. Solov'ev, Vestn. Akad. Med. Nauk SSSR, No. 2, 78 (1967).
7. A. Augustin and H. Cosenza, Eur. J. Immunol., 6, 497 (1976).
8. H. Binz and H. Wigzell, J. Exp. Med., 142, 197 (1975).
9. H. Binz and H. Wigzell, Nature (London), 264, 778 (1976).
10. H. Boehmer, J. Immunol., 112, 70 (1974).
11. P. Häyry and L. S. Andersson, Cell. Immunol., 17, 165 (1975).
12. N. K. Jerne, Ann. Immunol., 125, 373 (1974).
13. A. K. Kimura, J. Exp. Med., 139, 888 (1974).
14. T. J. McKearn, Science, 183, 94 (1974).
15. T. J. McKearn et al., J. Immunol., 139, 1876 (1974).
16. H. Ramseier, Eur. J. Immunol., 3, 164 (1973).
17. L. S. Rodkey, J. Exp. Med., 139, 712 (1974).
18. D. A. Rowley et al., Science, 181, 1133 (1973).

IMMUNOGENICITY AND THYMUS-DEPENDENCE OF POLYMERIZED *Clostridium perfringens* α-TOXOID

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By condensing the α-toxoid of Clostridium perfringens type A with glutaraldehyde a polymer of the α-toxoid with molecular weight 450,000-600,000 was obtained. Experiments on guinea pigs showed that the immunogenicity of both the monomer and the polymer of the α-toxoid, when used in the adsorbed form is practically identical. On immunization with unadsorbed antigens the primary response to the polymer was 3 times greater than the immune response to the monomer. Polymerization of the α-toxoid did not change its thymus dependence.

KEY WORDS: α-toxoid; polymer; immunogenicity; thymus dependence

A central place in the problem of the prevention of gas gangrene caused by Clostridium perfringens type A is occupied by the development of methods of obtaining highly immunogenic toxoid preparations. There are reports in the literature that the immunogenic properties of the natural antigens of aggregations of molecules can be enhanced by their covalent-bonding by chemical bridges [4, 8-10]. One method of polymerizing proteins is by condensing them with glutaraldehyde.

The object of this investigation was to obtain a polymer of the α-toxoid of Cl. perfringens by using glutaraldehyde and to study its properties.

EXPERIMENTAL METHOD

The α-toxoid was obtained by detoxication of the principal isocomponent of the α-toxin of Cl. perfringens type A strain BR6K No. 28. The toxin was concentrated by precipitation at the isoelectric point with increased ionic strength of the culture fluid and fractionated by the batch method on DEAE-cellulose, equilibrated in 0.005 M KH_2PO_4 solution [1]. Under these conditions the principal component of the α-toxin, accounting for 95% of the total activity of the α-toxin in the culture fluid, possesses negative adsorption. The α-toxin was

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