NATURE OF SERUM INHIBITORY FACTOR OF MICE INJECTED WITH ISOLOGOUS ANTIBODIES CONJUGATED WITH CELLULOSE

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It is now generally accepted that autologous anti-idiotypic reactions can regulate the immune response to antigen [2, 6-9, 12, 13]. The writers found previously that injection of syngeneic immunoglobulin, containing antibodies against sheep's red blood cells (SRBC), conjugated with cellulose, inhibited the immune response of these mice to SRBC. The sera of such mice, unlike the sera of mice receiving isologous immunoglobulin without cellulose, inhibited syngeneic antibody-forming cells (AFC) producing antibodies against SRBC but not against rat red blood cells (RRBC) [3]. It was suggested that the inhibitory activity of the sera studied was due to anti-idiotypic antibodies.

In the investigation described below, to test this hypothesis the sera for study were absorbed with immunoglobulins of varied specificity, conjugated with cellulose.

EXPERIMENTAL METHOD

Male (CBA × C57BL/6)F_1 ($H\text{-}2^{k/b}$) mice weighing 18-20 g were used. Antiserum against SRBC and antiserum against RRBC of the August line were obtained from animals immunized 3 times with 5×10^8 SRBC or RRBC, with intervals of 2 weeks [3]. Total globulins were isolated from normal mouse serum and from mouse antisera against SRBC or RRBC by precipitation twice with $(NH_4)_2SO_4$ at 50% saturation, followed by dialysis against 0.85% NaCl for 3 days at 4°C. Rabbit antibodies against mouse γ-globulin (AB-anti-MGG) used in the work were generously provided by E. V. Sidorova (N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR). Mouse antibodies against SRBC, eluted from erythrocytic sorbents, fixed beforehand with 0.1% glutaraldehyde, were obtained by the method described previously [4].

Activity of eluted antibodies (el-AB) was tested in the passive hemagglutination test. Their hemagglutination titer varied from 0.5 to 15 μg protein. Immunosorbents on a cellulose basis were obtained by the method in [1]. Immunosorbents immobilized by total globulin, containing antibodies against SRBC (Ig-anti-SRBC-tot) or against RRBC (Ig-anti-RRBC-tot), with eluted antibodies (el-AB-tot), with total normal globulin (norm-Ig-tot) and with rabbit antibodies against mouse γ-globulin (AB-anti-MGG-tot) were used. The protein concentration in the solution was determined by the SS-26 spectrofluorometer. The quantity of protein immobilized by the sorbent was determined as the difference between the quantity of protein in the initial solution and its amount in the supernatant after immobilization and in the washings.

To increase the immunogenicity of the protein antigens [5] (immunoglobulin or el-AB) they were conjugated with cellulose, and the animals were immunized with this conjugate. To obtain sera the following scheme of immunization of the animals was used: mice of group 1 were injected with Ig-anti-SRBC-tot (0.2 mg protein per mouse) followed 1 month later by Ag-anti-SRBC (0.2 mg protein per mouse); mice of group 2 were injected with el-AB-tot (0.1 mg protein per mouse) followed 1 month later by el-AB (0.034 mg protein per mouse); mice of group 3 received two injections of el-AB at an interval of 1 month (the first injection of 0.1 mg protein per mouse, the second injection 0.034 mg protein per mouse). All injections were given subcutaneously, at two points in the back. Sera were taken from the experimental

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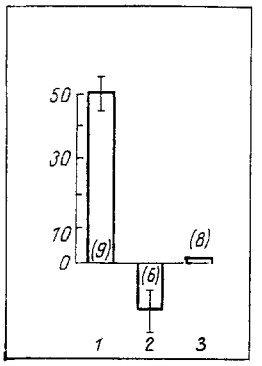

Fig. 1. Effect of sera of different groups of mice on number of 19S AFC producing antibodies against SRBC. Abscissa: 1-3) groups of mice, number of animals in group given in parentheses. Mouse sera tested individually; ordinate, reduction in number of AFC (in %). Group 1) Sera of mice receiving first injection of el-AB-tot and second injection of el-AB; group 2) sera of mice receiving two injections of el-AB; group 3) sera of normal mice.

mice on the 7th-8th day after the last immunization. To remove immune complexes [10], some sera of the mice of group 1 were subjected to ultracentrifugation at 110,000g for 2 h and the top third of the volume of the supernatant was studied. Absorption of the sera by different immunosorbents was carried out during 1 h at room temperature. For this purpose, 0.4 ml of test serum (dilution 1:2) was added to 0.4 ml of an immunosorbent containing 1 mg protein in 1 ml. After absorption the sera were centrifuged (2500 rpm for 5 min) and their effect on cells immune to SRBC was studied. For this purpose, spleen cells of mice immunized with 5×10^8 SRBC 4 days before the experiment (0.3 ml, 10^7 cells/ml) were treated with 0.1 ml of the test serum (dilution 1:2) and 0.1 ml of rabbit complement nontoxic for mouse cells (RC; dilution 1:2). The mixture was incubated at 37°C for 45 min, the cells were washed off by centrifugation (1500 rpm, 10 min), and the number of AFC against SRBC was determined by the local hemolysis in gel test. On the basis of the results of these experiments the percentage reduction in the number of AFC was calculated: $a - b/a \times 100\%$, where a is the number of AFC after incubation of the cell suspension with sera of intact mice in the presence of RC, and b the number of AFC after incubation of the cell suspensions with the test sera in the presence of RC.

EXPERIMENTAL RESULTS

Sera of mice receiving the first injection of el-AB-tot and a second injection of el-AB depressed by 50%, in the presence of RC, the number of AFC producing antibodies against SRBC (Fig. 1), in the same way as was shown previously for Ig-anti-SRBC-tot [3]. Sera of mice receiving two injections of el-AB did not inhibit AFC formation against SRBC under the same conditions (Fig. 1).

Since conjugation of a protein with cellulose is known to increase its immunogenicity [5], we suggested that in response to injection of idiotype-positive molecules conjugated with cellulose, anti-idiotypic antibodies would appear in the serum of the mice and would bring about inhibition of AFC against SRBC. To continue the study of the nature of the serum inhibitory factor, the test sera were adsorbed with immunoglobulins of varied specificity, conjugated with cellulose. Data showing the effect of these sera of AFC against SRBC before and after such absorption are given in Fig. 2. Before adsorption the test sera inhibited AFC producing antibodies against SRBC by 75%. Absorption of the sera by Ig-anti-SRBC-tot, el-AB-tot, and AB-anti-MGG-tot immunosorbents abolished their inhibitory activity, unlike absorption with Ig-anti-RRBC-tot and norm-Ig-tot immunosorbents. The top third of the volume of the ultracentrifuged (at 110,000g for 2 h) test sera had the same action on AFC against SRBC as whole sera not subjected to ultracentrifugation (data not shown).

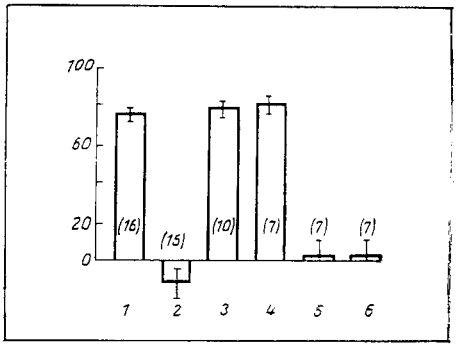

Fig. 2. Changes in activity of sera from mice receiving injections of Ig-anti-SRBC-tot and Ig-anti-SRBC as a result of absorption by various immunosorbents. Abscissa: 1-6) groups of mice, number of sera tested given in parentheses; ordinate, inhibition of AFC (in %). Group 1) Sera before absorption, groups 2-6) sera after absorption by Ig-anti-SRBC-tot, norm-Ig-tot, Ig-anti-RRBC-tot, el-AB-tot, and AB-anti-MGG-tot respectively.

On the basis of these results it can be concluded that the inhibitory activity of the sera of mice receiving a first injection of Ig-anti-SRBC-tot (or el-AB-tot) and a second injection of Ig-anti-SRBC (or el-AB respectively) is due to antibodies, for it was absorbed by AB-anti-MGG-tot. This inhibitory activity was evidently not due to a complex of the initial antigen (SRBC) with antibodies against SRBC for, first, SRBC were not injected into the mice producing the test serum and, second, the top third of the volume of the ultracentrifuged sera (which did not contain antigen-antibody complexes) induced the same reduction in the number of AFC as whole nonultracentrifuged serum. In the writers' opinion, the serum inhibitory factor is anti-idiotypic in nature, for it was absorbed by Ig-anti-SRBC-tot and el-AB-tot, i.e., by immunosorbents with idiotype-positive molecules, but not by Ig-anti-RRBC-tot or norm-Ig-tot. Further evidence of the anti-idiotypic nature of the antibodies also is given by the writers' previous data [3] showing that the inhibitory activity of the serum after injection of Ig-anti-SRBC-tot and Ig-anti-SRBC is found in normal and T-B mice (B mice injected with thymocytes), but is not observed in B mice. This is in in agreement with conclusions regarding T-dependence of anti-idiotypic antibody production [11].

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