CYTOTOXIC SERUM AGAINST B LYMPHOCYTES PREPARED BY IMMUNIZATION WITH BONE MARROW CELLS

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By immunization of rabbits with bone marrow cells of intact mice antisera reacting selectively with cells of bone marrow origin are obtained. The method of obtaining anti-B sera by immunizing animals directly with bone marrow cells makes it unnecessary to subject the mice to irradiation or thymectomy, which are necessary when methods described previously are used.

KEY WORDS: *anti-B serum*; *bone marrow*.

To study the principles of development of the immune response under experimental and clinical conditions it is essential to be able to detect B and T lymphocytes in the organs and tissues and to evaluate the processes of their cooperation and the possibility of selective attack on the two types of lymphocytes. For these purposes specific anti-T and anti-B sera are widely used.

Preparation of anti-B sera by existing methods [6-8] meets with certain difficulties because cells from lymph nodes [8] or the spleen [6, 7] of specially prepared mice are used for immunizing rabbits. The preparation consists of thymectomy and lethal irradiation of the mice, followed by the repopulation of their lymphoid organs with syngeneic embryonic liver cells [8] or bone marrow cells [6, 7]. It is assumed that the lymph node or spleen cells used for immunization contain chiefly a population of B cells. However, the resulting sera have to be reabsorbed by mouse tissue antigens and repeatedly absorbed by thymocytes [6-8].

Since bone marrow contains an extensive population of B cells and virtually no T lymphocytes [1], in this investigation an attempt was made to use bone marrow cells directly as the antigen for producing anti-B serum.

EXPERIMENTAL METHOD

The donors of the bone marrow cells were noninbred female albino mice weighing 16-18 g. Cells for immunization were obtained from the sternum by mincing it with scissors and also from the bone marrow of the femur and tibia with the aid of an injection needle and syringe.

To remove contaminating red cells, the cells were treated with 0.83% ammonium chloride solution in Tris buffer [2] and then washed at least 4 or 5 times with physiological saline. The washed cells, in a concentration of $170 \cdot 10^6$, were injected intravenously into rabbits weighing 2-2.5 kg on 3 successive days, followed by an interval of 4 days; these cycles were repeated for 3 weeks. During the course of immunization of one rabbit, from 65 to 70 mice were required as donors of bone marrow cells.

Antisera obtained 1 week after the last immunization were heated to 56°C for 30 min and absorbed initially with mouse liver homogenates and mouse erythrocytes in the proportion of 1 ml serum to 0.1 ml of the solid residue of each type of cells, twice, for 1 h each time, at room temperature. Frequent absorption with mouse thymocytes was then carried out in the same way in order to remove the residual antithymus activity from the sera. After absorption the antisera were tested for cytotoxicity against cells of the thymus, lymph nodes, spleen,

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and bone marrow of CBA mice and they were also investigated for syngeneic transfer of their ability to inhibit antibody-forming cells (AFC) and to produce humoral antibodies.

The cytotoxicity test was carried out in plastic panels by a modified method of Niederhuber and Möller [7]. The cells ($2.5 \cdot 10^7$ in 1 ml) in Earle's solution were mixed with immune or normal serum and incubated at 37°C for 15 min. Complement was then added in a dilution of 1:3 to the mixture of cells with serum in each well. Fresh guinea pig serum, absorbed by sheep's red cells and homogenates of mouse liver and spleen, was used as complement. Absorption was carried out at room temperature in the same way as the absorption of complements by agarose [3]. All ingredients were used in a volume of 0.1 ml.

The panel was placed on ice 45 min after addition of the complement, 0.3 ml of a 0.2% aqueous solution of trypan blue was added to each well, and 5 min later the living and dying cells were counted in a Goryaev chamber. The cytotoxic indices were determined, in percent, by the formula [7, 8]:

$$\frac{\%\text{ of dying cells in mixture with antiserum} - \%\text{ of dying cells in mixture with normal serum}}{100 - \%\text{ of dying cells in mixture with normal serum}}$$

In the adaptive transfer experiment CBA mice were immunized intravenously with sheep's red cells in a dose of $1 \cdot 10^9$, washed 5 times with physiological saline. Two weeks later the spleen cells of these animals were treated for 1 h at 37°C with absorbed antibone-marrow serum or normal rabbit serum respectively in the presence of guinea pig complement. For this purpose, the cells, serum, and complement were mixed so that the last two ingredients were in a dilution of 1:10. After incubation the cytotoxic index of the antiserum was calculated, the cells were washed twice with cold Earle's solution at 1200 rpm for 5 min, and they were injected intraperitoneally in a dose of 2×10^7 cells into syngeneic mice, treated the day before by intraperitoneal injection of cyclophosphamide in a dose of 4 mg per mouse. Four days later the number of direct and indirect AFC was determined among the spleen cells of the recipient mice and their number per spleen was calculated. Direct AFC were detected by the method of Jerne and Nordin [5] and indirect by the method of Dresser and Wortis [4]. Rabbit serum (1:80) against mouse IgG, obtained by the use of caprylic acid [9], was used to reveal indirect AFC.

To investigate AFC in both the experimental and control groups, mixtures of homogenates of three spleens were used. Each group consisted of 18 mice. The content of hemolysins in the sera was determined individually in each mouse.

EXPERIMENTAL RESULTS

As Table 1 shows, the sera against bone marrow cells, after absorption by mouse liver homogenate and mouse red cells, did not acquire the ability to react selectively with bone marrow cells only, but continued to react also with thymocytes. After exhaustion with thymocytes, the sera no longer had any cytotoxic action on them. The cytotoxicity of the antisera against lymph node and spleen cells was reduced by more than half, and against bone marrow cells by 19% (by only 8% with the sera in a dilution of 1:100).

Treatment of the immune spleen cells with antibone-marrow serum in the presence of complement led to death of 62% of the lymphocytes. There was a corresponding sharp decrease in the number of AFC in the spleen and in the blood hemolysin levels of the animals. Only 52 ± 2.6 direct and 55 ± 5.3 indirect AFC were found in the recipients' spleen compared with 1950 ± 106 and 5150 ± 122 in the control with normal serum ($P < 0.001$). The hemolysin titer was lowered to 18.5 ± 2.3 (in the control 345.6 ± 38.2; $P < 0.001$).

The absence of reaction of the absorbed sera with thymocytes and the considerable decrease in their ability to exert a cytotoxic action on lymph node and spleen cells were probably mainly attributable to removal of antibodies reacting with θ antigen. Since the number of such cells in bone marrow is negligible [1], most bone marrow cells remained capable of reacting with serum.

The marked decrease in the number of AFC in the spleens and in the hemolysin titer in the sera of the mice after reaction of the immune spleen cells with antibone-marrow serum is evidence of the cytotoxic action of this serum on the antibody producers. Under these circumstances both cells producing IgM and cells producing IgG antibodies were equally inhibited.

The question of whether antisera against bone marrow cells can react with stem cells still remains unanswered. All that can be said is that the cytotoxic indices of sera obtained

TABLE 1. Indices of Cytotoxicity of Sera against Mouse Bone Marrow Cells before and after Absorption by Thymocytes

Source of cells	No. of serum	Index of cytotoxicity of sera			
		before absorption by thymocytes		after absorption by thymocytes	
		1:10	1:100	1:10	1:100
Thymus	1	55±4,97	17±3,76	0	0
	2	55±4,97	31±3,27	0	0
	3	100	78±2,93	0	0
	4	97±1,71	57±3,50	12±2,29	6±1,68
Lymph nodes	1	63±2,04	24±3,43	15±3,02	8±2,29
	2	64±4,28	45±3,67	35±3,37	33±3,32
	3	90±2,67	58±4,93	30±3,24	22±2,93
	4	70±4,08	45±3,81	48±3,53	24±3,02
Spleen	1	49±4,13	19±3,37	38±4,85	5,8±2,34
	2	77±4,21	54±3,94	20±2,83	11±2,21
	3	78±4,14	46±2,98	21±2,88	20±2,83
	4	83±3,76	65±2,90	40±3,46	38±3,43
Bone marrow	1	61±3,36	37±3,36	56±4,96	37±4,82
	2	57±4,28	53±4,07	49±3,53	40±3,46
	3	80±3,45	46±2,98	39±3,45	25±3,06
	4	73±3,83	46±3,39	51±3,53	49±3,53

Note. Numbers of sera correspond to numbers of rabbits. Survival rate of cells in mixture with normal serum (control) was 80-90% in all experiments.

by other workers [6-8] using lymph node and spleen cells did not differ from the indices of our own sera.

The inequality of the intensity of the residual antithymus activity of sera obtained from different rabbits is an interesting fact. In some cases, in order to completely suppress cytotoxic activity against thymocytes, the serum needed to be absorbed with thymocytes for 1 h at a time for only 4 times, but in other cases for 9 times. Occasionally even after 12 repeated absorptions the antiserum was still able to cause death of 12% of the thymus cells.

According to Niederhuber [6], the residual antithymus activity of anti-B sera is due to the IgM which they contain. The antithymus activity of the sera obtained in the present experiments may perhaps also be explained by contamination with different quantities of IgM.

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