SUPPRESSOR ACTION OF AUTOLOGOUS THYMOCYTES ABOLISHED BY PREDNISOLONE

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The action of autologous thymocytes on the formation of hemolysin-producing cells was investigated in various lymphoid formations in young rabbits. Living thymocytes, injected intravenously, were shown to inhibit the formation of antibody-forming cells in the animals' spleen. Treatment of these cells before injection with prednisolone abolished the depressor effect of the autologous thymocytes.

KEY WORDS: immune response; immunosuppression; suppressor thymocytes; prednisolone.

In the modern view lymphocytes with suppressor action play a particularly important role in the regulation of immunogenesis at the cell level. Such cells are found in the peripheral blood, spleen, and thymus [1-3, 5]. Experiments in vitro have shown that syngeneic thymocytes can inhibit both antibody formation and reactions of cellular immunity [2, 4, 8, 9].

In this investigation the effect of intact and prednisolone-treated autologous thymocytes on the formation of hemolysin-producing cells was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 39 young (aged 5 months) rabbits weighing about 2-2.5 kg. Under pentobarbital anesthesia (30 mg/kg) about one-quarter of the thymus was removed from the animals and a suspension of thymocytes was immediately prepared in double Eagle's medium as the nutrient medium. The original proportion of viable cells in the trypan blue test was 85-95%. Thymus lymphocytes washed with Eagle's medium (10^8 or 10^7 cells in 1 ml) were incubated for 1 h at 37°C, washed again with the above-mentioned medium, and injected intravenously into the animals in doses of 10^8, 10^7, and 10^6.

Some thymocytes were incubated with medium containing prednisolone in a concentration of 500 μg/ml. After incubation for 1 h at 37°C the thymocytes were washed with Eagle's medium and injected into the animals in a dose of 10^7-10^8 cells. Preliminary experiments to choose the prednisolone concentration showed that the concentration mentioned above does not significantly increase the number of nonviable cells compared with the control, whereas a further increase in the prednisolone concentration was accompanied by considerable mortality of the thymus lymphocytes 1 h after culture of these cells with prednisolone under thermostatically controlled conditions.

Intact rabbits, animals from which one-quarter of the thymus was removed, and rabbits receiving injections of thymus cells killed by heating (30 min at 60°C) formed the control group. The animals were immunized immediately after the injection of thymocytes by intravenous injection of 10^9 sheep's erythrocytes (SE) (2.5 kg body weight). Antibody-forming cells (AFC) were determined by the method of Jerne and Nordin [6] on the 5th day in the spleen, tonsils, submandibular lymph nodes, and appendix. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. They show that as a result of injection of 10^6-10^8 thymocytes the number of AFC fell significantly, mainly in the spleen tissue. The reason for this was evidently that most of the thymocytes are concentrated in the spleen after intravenous injection, as experiments with thymocytes labeled with ^{51}Cr showed (Table 2).

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TABLE 1. Number of AFC in Lymphoid Organs of Rabbits Immunized with SE

Group No.	Number of animals	Number of AFC (per 10^6 cells) in			
		spleen	tonsils	appendix	lymph nodes
1	4	130,0±25,0	46,3±6,2	10,5±4,7	17,2±4,7
2	4	165,0±57,5	35,0±12,5	11,0±2,0	18,2±1,8
3	3	183,3±55,3	25,6±1,3 $P<0,05$	4,0±2,4	12,0±1,0
4	9	36,3±7,8 $P<0,05$	36,4±9,8	11,5±2,3	9,2±3,9
5	4	56,5±16,5 $P<0,05$	26.7±12,8	7,0±4,7	17,5±4,7
6	3	44,3±16,2 $P<0,05$	24,3±12,8	5,3±3,1	9,3±2,0
7	4	143,0±57,7	50,0±13,8	13,2±3,8	9,0±4,8
8	5	110,0±47,8	21,2±8,8	14,2±6,0	12,0±2,0

Legend. Values of P given for significant differences from control (group 1). Details of groups: 1) intact rabbits immunized with SE; 2) animals immunized with SE after removal of one-quarter of thymus; 3) animals immunized with SE after intravenous injection of killed autologous thymocytes; 4, 5, 6) rabbits immunized with SE after intravenous injection of 10^8, 10^7, and 10^6 living autologous thymocytes respectively; 7, 8) rabbits immunized with SE after intravenous injection of 10^8 and 10^7 autologous thymocytes, treated with prednisolone, respectively.

TABLE 2. Radioactivity of 100 mg Tissue from Various Lymphoid Formations of Rabbits 20 h after Intravenous Injection of Autologous Thymocytes Labeled with ^{51}Cr

Organ	Radioactivity, cpm			
	background	rabbit No. 1	rabbit No. 2	rabbit No. 3
Spleen	30	1500	700	2000
Tonsils	24	250	240	300
Appendix	24	180	160	250
Thymus	18	250	280	210
Lymph nodes	26	240	200	350
Radioactivity of original 10^8 autologous thymocytes		10^6	$5\cdot10^5$	10^6

Injection of dead thymus cells or removal of one-quarter of the mass of the thymus had no significant effect on the indices of antibody formation. Meanwhile, treatment of the thymocytes with prednisolone abolished the suppressor action of the autologous thymocytes.

The results thus indicate that thymocytes can inhibit antibody formation in vivo. The depressor activity of these cells may be manifested either as the result of a simple excess of thymocytes or as a result of the presence of specialized suppressor cells in the thymus [5, 7].

Abolition of the depressive action of autologous thymocytes by preliminary treatment with prednisolone has nothing to do with death of the cells and may be indirect evidence that the thymocyte population is functionally heterogeneous and contains depressor sensitive to the action of prednisolone.

Abolition of the suppressor activity of the thymocytes by prednisolone evidently falls into the category of biologically purposive reactions leading to an increase in immunologic reactivity in stressor situations.

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EFFECT OF ANTIBODIES AGAINST DENATURED DNA ON HUMAN BONE MARROW CELLS FORMING COLONIES IN SEMISOLID AGAR

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γ-Globulin isolated from rabbit sera containing antibodies against denatured DNA or cytidine reduces the efficiency of colony formation in agarized cultures of human bone marrow. Antibodies against DNA isolated from immune sera by means of an immunoadsorbent possess a similar action. After removal of antibodies against denatured DNA from the sera of intact animals and immune sera, the γ-globulin from these sera in a concentration of 0.28 and 5 mg per $2 \cdot 10^5$ explanted nucleated cells had no effect on colony formation, but if added in a dose of 15 mg stimulated growth of the colonies.

KEY WORDS: antibodies against DNA; colony formation; bone marrow cells.

Antibodies against DNA are found in the sera of patients with various collagen diseases [2, 3, 11]. Existing information on the ability of antibodies to prevent DNA from performing its template function [1] suggests that antibodies against DNA may play a definite role in the development of the leukopenia observed in these diseases. It was shown previously that artificially induced antibodies against denatured DNA can depress the development of splenic endogenous colonies in mice irradiated in sublethal doses [4]. However, since irradiation has an additional action on the organism and, in particular, it modifies DNA metabolism there is good reason to investigate the action of antibodies on proliferating hematopoietic cells in experiments in vitro.

In this investigation the action of γ-globulin from rabbit sera containing antibodies reacting with DNA on human hematopoietic cells capable of forming colonies in semisolid agar was studied.

EXPERIMENTAL METHOD

DNA from calf thymus was obtained by the method of Kay et al. [7] with additional deproteinization by chloroform and isoamyl alcohol. The DNA (400 μg/ml) was denatured by heating to 100°C for 10 min and then cooling in an ice bath.

The methods of obtaining antisera against denatured DNA, of preparing the immunoadsorbent with DNA denatured in the presence of formaldehyde, and of adsorption of the antibodies from the immune sera were described previously [4].

Sera with an antibody titer of 1/1280-1/2560, determined by the passive hemagglutination test [3], were used. Pure antibodies were obtained by elution with 2.5 M $MgCl_2$ from the immunoadsorbent, previously washed with standard salt solution (0.15 M NaCl and 0.015 M sodium citrate). Antibodies against cytidine were obtained by Erlanger's method [6]. The γ-globulin was precipitated from the sera with $(NH_4)_2SO_4$ at 40% saturation, dialyzed against 0.15 M NaCl to remove all the $(NH_4)_2SO_4$, and then dialyzed against Hanks' medium.

The protein content was determined by Lowry's method [8] and spectrophotometrically at 280 nm. The preparations were sterilized by passage through Synpor 6 nitrocellulose membrane filters (Czechoslovakia) and made up to equal protein concentration with McCoy 5A medium, and then added to the cultures. Culture

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