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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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TABLE 1. Action of γ-Globulin from Sera Containing Antibodies against Denatured DNA and Cytidine, and also "Pure" Antibodies against DNA on Colony Formation in Agarized Cultures of Human Bone Marrow

Acting factor	Experiment 1			Experiment 2			Experiment 3		
	protein content per $2 \cdot 10^5$ cells, mg	mean number of colonies per 10^5 cells	P	protein content per $2 \cdot 10^5$ cells, mg	mean number of colonies per 10^5 cells	P	protein content per $2 \cdot 10^5$ cells, mg	mean number of colonies per 10^5 cells	
γ-Globulin with antibodies against DNA	5	15±1,0	<0,001	15	49±1,4	<0,001	15	26±3,3	<0,01
γ-Globulin with adsorbed antibodies	—	—	—	15	81±1,7	>0,05	15	50±1,4	>0,05
γ-Globulin with antibodies against cytidine	—	—	—	15	60±2,8	<0,01	15	31±2,8	<0,01
"Pure" antibodies against DNA	—	—	—	—	—	—	0,28	32±2,8	<0,01
McCoy 5A medium	—	30±2,0	>0,05	—	58±1,4	<0,01	—	42±4,3	<0,05
γ-Globulin from intact rabbit sera	5	32±1,2	—	15	82±1,4	—	15	56±4,3	—
The same	—	—	—	—	—	—	0,28	40±4,3	<0,05

Legend. P calculated by comparison with cultures to which γ-globulin from intact rabbit serum was added.

of the cells in agar was carried out in glass Petri dishes 60 mm in diameter by the method of Pike and Robinson [9], by means of which precursor cells of human granulocytes can be cloned.

Peripheral blood leukocytes from donors in the bottom layer of the nutrient medium with 0.5% agar in a concentration of 10^6/ml were used as the colony-stimulating factor. After solidification of the agar, bone marrow cells were placed on the bottom layer in a concentration of $1 \cdot 10^5$-$2 \cdot 10^5$/ml nutrient medium. The agar concentration in the top layer was 0.3%. The cells were cultured at 37°C in an atmosphere of air mixed with 10% CO_2.

The colonies were counted on the 10th-12th day of culture under a magnification of 70×. Groups of 50 cells or more were regarded as colonies.

The statistical analysis was carried out by Student's method [5].

EXPERIMENTAL RESULTS

The results of three independent experiments to study the effect of different quantities of antibodies against DNA and cytidine on the efficiency of colony formation (ECF), defined as the number of growing colonies for every 10^5 explanted nucleated cells, are given in Table 1. The mean ECF without the addition of γ-globulin was 30.0, 58.0, and 42.0. In all three experiments an inhibitory action of antibodies against DNA on ECF was found. Addition of 5 mg immune γ-globulin to the culture was found to have a greater effect than addition of 15 mg. The explanation of this phenomenon may be that addition of γ-globulin from intact rabbit sera to the culture in the proportion of 15 mg to $2 \cdot 10^5$ cells leads to a significant increase in ECF ($P < 0.05$) compared with cultures without the addition of γ-globulin. Immune γ-globulin, when added in higher concentrations, thus evidently had a twofold action: inhibitory on the one hand and stimulating on the other. γ-Globulin from sera from which antibodies against DNA had been removed gave a similar effect.

Additional data indicating the ability of antibodies against DNA to affect the proliferation of bone marrow cells were obtained by the use of "pure" antibodies against DNA.

Antibodies against DNA obtained in response to the DNA molecule in the form of a complex with methylated bovine serum albumin are known to belong to the IgM class [11]. Antibodies against DNA from patients with collagenoses can belong to both IgM and IgG classes [11]. To investigate the effect of the class of immunoglobulins on ability to depress ECF, antibodies induced by injection of cytidine conjugated with bovine serum albumin were used in two experiments. The antibodies thus obtained, which belonged to both IgM and IgG classes [10], also had an inhibitory effect on ECF.

The results are thus evidence that antibodies against DNA and cytidine depress colony formation in agarized cultures of human bone marrow and they confirm the view that rapidly dividing cells with intensive DNA metabolism may be the target for the action of such antibodies.

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