EFFECT OF ANTIBODIES AGAINST DNA ON HEMATOPOIETIC STEM CELLS

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Injection of rabbit sera containing antibodies against denatured DNA reduces the number of endogenous splenic colonies in mice irradiated in a dose of 600 rad. The serum of intact animals and antiserum from which the antibodies against denatured DNA were removed by an immunosorbent have no such effects. The results indicate that antibodies against denatured DNA may suppress the proliferation and differentiation of hematopoietic stem cells.

Antibodies against various structures of DNA are found in the blood serum of patients with systemic lupus erythematosus [3, 6]. A soluble complex of DNA and antibodies against DNA is deposited in the basement membrane and mesangium of the glomeruli and probably plays a role in the development of lupoid glomerulonephritis [10]. No evidence has been obtained of a pathogenic action of the antibodies directly on the body cells. Nevertheless, considering the important role of nucleic acids in the vital activity of the organism it is logical to assume that such a possibility exists, because antibodies against DNA could conceivably bind with certain sites on the nucleic acid and prevent normal biochemical processes from taking place in various cells.

In the investigation described below the action of animal sera containing antibodies reacting with denatured regions of DNA on the stem cells of hematopoietic tissue was studied. It was assumed, when this model was chosen, that rapidly dividing cells with intensive DNA synthesis might be sensitive to the action of the antibodies.

EXPERIMENTAL METHOD

DNA was obtained from calf thymus by the method of Kay et al. [9] with additional deproteinization with chloroform and isoamyl alcohol. The DNA (concentration 500 μg/ml) was denatured in the presence of 1.3% formaldehyde by heating to 100°C for 10 min.

TABLE 1. Change in Number of Exogenous Splenic Colonies after Three Injections of Immune Serum

Treatment on 3rd, 4th, and 5th days after irradiation	Antibody titer in PHT	Number of mice	Mean number of endocolonies per spleen	95% confidence interval	P_1
Immune serum	1/10000	15	8,0	5,9±10,8	<0,05
Immune serum exhausted with immunosorbent	1/10	15	12,8	10,1—16,1	>0,05
Immune serum treated with cellulose	1/5000	15	8,2	5,6—12,1	<0,05
Serum of intact animal	1/10	15	10,4	8,3—13,1	>0,05
Physiological saline	—	15	11,2	8,5—14,8	—

P^1 — significance of differences from control

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Combined immunization of rabbits with DNA and methylated bovine serum albumin (MBSA) in Freund's complete adjuvant was carried out in the plantar pads and subcutaneously by three injections at weekly intervals [11]. Ten days after the last injection the DNA–MBSA complex was injected intravenously. This intravenous injection of the complex was repeated monthly. The appearance of antibodies against DNA in the sera of the immunized animals and their content were estimated from the results of the passive hemagglutination tests (PHT) [4]. Since large quantities of antibodies were required simultaneously for these experiments, sera taken from several immunized animals were pooled.

In the control series sera with adsorbed antibodies were used. To remove the antibodies from the immune sera an immunosorbent, prepared as follows, was used. Previously washed DEAE-cellulose was suspended in standard salt solution (0.15 M NaCl and 0.015 M sodium citrate), packed into a column, saturated with DNA denatured in the presence of formaldehyde, and washed with the standard salt solution. The immunosorbent was added to the immune serum and the mixture incubated for 1 h at room temperature with careful mixing. The immunosorbent was then removed by centrifuging. Completeness of exhaustion of the antibody was checked by the PHT.

The effect of anti-DNA antibodies on hematopoietic stem cells was studied by the splenic endocolony method [14]. Noninbred albino mice aged about 3.5 months were irradiated with γ rays on the Luch-1 in a dose of 600 rad with a dose rate of about 50 rad/min. Sera, inactivated by heating to 56°C, with and without anti-DNA antibodies were injected in a volume of 0.25 ml on the 3rd, 4th, and 5th days after irradiation. The mice were killed 9 days after irradiation and the spleens were removed and fixed in a mixture of ethanol and glacial acetic acid (3:1). After fixation for 2-3 h the total number of endogenous splenic colonies more than 0.4 mm in diameter was counted. Since the distribution of the number of endocolonies among the spleens was close to lognormal in character, statistical analysis of the experimental results was carried out by the method of Smith et al. [13].

EXPERIMENTAL RESULTS

The results of one of the experiments to investigate splenic endocolonies in mice after three injections of anti-DNA antiserum are given in Table 1. A statistically significant decrease in the number of endogenous splenic colonies was found in the animals receiving sera containing antibodies against denatured DNA compared with the group of mice not receiving the sera. The serum of intact animals and adsorbed immune serum had no inhibitory effect. Consequently, the decrease in the number of endocolonies by the action of the immune sera was due to the action of anti-DNA antibodies and not to a switch of hematopoiesis in the mice to lymphopoiesis, when no colonies are formed in the spleen [7].

Since DNA fixed on DEAE-cellulose, which can adsorb some serum proteins, was used as the immunosorbent, antiserum treated with DEAE-cellulose as well as with the immunosorbent was used as the carrier control. In this case the titer of anti-DNA antibodies in the immune sera either remained unchanged or fell very slightly. Antisera treated in this way had almost the same inhibitory action as the original antisera.

A decrease in the number of splenic colonies also was observed after a single injection of antibodies on the 3rd day after irradiation, although the effect in this case was less marked.

The results of these experiments thus show that anti-DNA antibodies can inhibit the proliferation and differentiation of hematopoietic stem cells. Considering that anti-DNA antibodies inhibit the synthesis of DNA and RNA in purified DNA- and RNA-polymerase systems [1, 5, 15] and in isolated mammalian cell nuclei [1], it can be postulated that the disturbance of these processes is actually responsible for inhibition of growth of the stem cells. An essential condition for the materialization of this possibility is direct contact between antibodies and DNA. Antipurine and antipyrimidine antibodies are known to penetrate into fertilized sea urchin eggs and to inhibit their development [12]. Meanwhile, Liebeskind et al. [8] found that after adding antithymine antibodies to a culture of normal Chinese hamster lung tissue cells no antibodies were found in the cells and they did not prevent development of the cells. However, antithymine antibodies do penetrate into malignized cells of this tissue and do inhibit their growth. The possibility cannot be ruled out that antibodies against DNA can also penetrate into some normal fast-dividing cells and, in particular, into hematopoietic stem cells.

There is another possible mechanism of inhibition. If it is accepted that breakdown products of DNA formed as a result of mass death of lymphocytes are reutilized and are essential for the development of the population of hematopoietic cells, the binding of these products with antibodies could be a cause of delay in the development of colonies in the spleen. It follows from the data in the literature [2] that liberation of

DNA breakdown products reaches its maximum in the first day after irradiation. This mechanism is therefore unlikely but experimental proof is essential.

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