ROLE OF IMMUNE COMPLEXES IN THYROID DISEASES

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Circulating immune complexes (CIC) are found in the blood of patients with autoimmune endocrine diseases, such as type I diabetes mellitus, and autoimmune thyroiditis, with a frequency of 50-90% of cases [2, 3, 5]. In diabetics the complexes persist for a long time in the blood and are sedimented on the walls of blood vessels, injuring them, a fact which points to participation of CIC in the pathogenesis of angiopathies [3, 5]. In autoimmune diseases of the thyroid gland, immunofluorescence studies have revealed deposition of immune complexes on microsomes of epithelial cells and in the colloid of the thyroid gland, but the mechanism of their action has not been studied [10]. The basic protein of the thyroid gland, namely thyroglobulin (TG), is not only the reserve form of the thyroid hormones, but it is also an antigen for the formation of antibodies to tri-iodothyronine (T_3) and thyroxine (T_4), whose importance in the clinical picture of the disease is disputed [4, 6-8]. CIC may perhaps block hormone formation as a result of interaction between antibodies and antigenic determinants of TG.

The aim of this investigation was to study the role of immune thyroglobulin–antibody (TG—AB) complexes in hormone formation by the thyroid gland. For this purpose, in experiments in vitro iodination of the TG—AB complex, of intact TG isolated from the bovine thyroid gland and the thyroid gland of a patient with nodular euthyroid goiter, and of partially purified AB to TG was carried out and the number of iodoamino acids formed (MIT, DIT, and T_4) per mole of TG in the immune complex was compared with the remaining samples.

EXPERIMENTAL METHOD

Pure TG was obtained from saline extracts of the thyroid gland of patients with euthyroid goiter and from healthy bovine thyroid glands by gel-filtration on Sephadex G-200. Homogeneous TG was obtained in the form of a single peak with sedimentation coefficient of 19S. Precipitating antibodies against TG with a titer of 1:32-1:64 were obtained by immunizing rabbits with human TG together with Freund's complete adjuvant, in the form of subcutaneous injections. Partially purified antibodies were isolated by precipitating the IgG fraction with 30% methanol at −5°C from the blood serum of the immunized animals. The TG—AB immune complex was obtained by mixing antiserum of rabbits with a solution of TG in concentration of 300-400 μg/ml, with determination of protein by Lowry's method in the washed precipitate. To determine the number of moles of antibodies bound with 1 TG molecule, the following equation was deduced:

$$\beta = 4.47\left(\frac{100-\alpha}{\alpha}\right),$$

where α denotes the percentage content of TG in the TG—AB complex, and β the number of moles of antibodies per mole of TG. The solutions of TG, antibodies, and the TG—AB immune complex in phosphate buffer, pH 9.0, were iodinated with a solution of KI + I_2 at 37°C for 30 min with mixing. The level of added iodine amounted to 200 moles I_2 per mole of protein. Monoiodotyrosine (MIT), di-iodotyrosine (DIT), and T_4 were determined quantitatively in the test proteins by the method in [1] on an EPC-3T spectrophotometer (Hiitachi). The molar content of the iodoamino acids was calculated by the recurrent rule.

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TABLE 1. Spectra of Iodoamino Acids of TG, TG—AB Immune Complex, and Antibodies during Iodination

Number of iodoamino acids/TG	Bovine TG		Iodinated bovine TG-AB	Human TG		Iodinated human TG–AB complex	Iodinated antibodies
	intact	iodinated		intact	iodinated		
MIT	9.9±0.1	14.7±0.7*	21.1±1.5*	4.1±0.7	7.5±0.8	11.0±1.1	6.1±0.7
DIT	11.8±1.1	19.7±1.7*	16.6±2.6	3.8±0.8	9.1±1.2*	5.1±0.9	1.0±0.5
T_4	2.6±0.1	4.0±0.6*	2.3±0.3	0.9±0.5	1.1±0.4	0.6±0.3	1.0±0.8

Legend: *) Statistically significant on comparison of spectra of iodinated TG and TG—AB complex with intact TG of the corresponding species. Number of experiments in all variants was 5.

EXPERIMENTAL RESULTS

The results show that TG isolated from different patients bind from 6-7 to 30-40 moles of antibodies per TG molecule. TG isolated from bovine thyroid gland bound 5 times less antibodies against human TG, evidence of the existence of one or several determinants, responsible for the species specificity of TG. Comparison of the results of spectral analysis of TG from the patients with the results of the precipitation test showed that the content of MIT in the protein was 3.8 ± 1.0, DIT 3.4 ± 0.8, and T_4 0.8 ± 0.1 per mole of TG, which altogether is several times less than the number of antibodies bound with a single molecule of TG, namely 20.1 ± 5.0.

During iodination of bovine TG a significant increase took place in the number of both MIT and DIT, as well as T_4. Similar results were obtained on iodination of the patient's TG (Table 1). A significant increase in the number of iodoamino acids on iodination of TG was not accompanied by any appreciable increase in binding of antibodies with modified TG. Nye and co-workers [9] obtained similar results.

Meanwhile, according to the present result, during immunochemical interaction of iodinated TG with antibodies an increase in binding of antibodies with antigen by 2-3 molecules, which corresponds to the number formed during iodination of T_4 molecules. Binding of antibodies with the antigenic determinants of thyroglobulin, namely T_3 and T_4, may evidently lead to loss of biological activity of the hormones, reduction of their secretion into the blood, and also competition for organically bound iodine between TC and the active centers of the antibodies [11, 12].

To obtain direct proof we iodinated the immune complex TG—AB and the spectra of the iodoamino acids thus formed were compared with the number of analogous iodoamino acids obtained by iodination of native TG and of antibodies to TG, isolated in the form of an IgG fraction. Mild iodination of the bovine and human TG—AB immune complex followed by its enzymic hydrolysis was accompanied by an increase in the total content of MIT and a decrease in the quantity of DIT and T_4 (Table 1). The increase in the MIT fraction after iodination of the complex may be linked with additional iodination of antibodies. In fact, partially purified antibodies to TG were synthesized only by MIT, amounting to 6.1 ± 0.7; DIT and T_4 were found only in traces: 1.0 ± 0.5 and 1.0 ± 0.8 respectively.

The results are thus evidence that binding of TG with antibodies into an immune complex leads to reduction of hormone formation by the thyroid gland. These processes evidently take place also in vivo in patients, and this is of great importance in the pathogenesis of thyroid diseases.

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EFFECT OF B LYMPHOCYTES FROM DIFFERENT ORGANS ON HEMATOPOIETIC COLONY FORMATION IN THE SPLEEN BY BONE MARROW CELLS

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T lymphocytes, activated by isoantigens, influence the process of hematopoietic colony formation in the spleen of irradiated recipients and alter the ratio between colonies of different types through their action on the control of differentiation of syngeneic hematopoietic cells [2]. B lymphocytes are involved in the realization of this effect, and serve as helper Tα-cells of the T lymphocytes realizing a differential effect [1]. Other forms of action of lymphoid cells and their products on colony formation also are known. It has been shown, for instance, that hematopoietic bone marrow precursor cells cannot form splenic colonies in the absence of precursors of T lymphocytes (PTL) or their products [4-6, 9]. It is interesting to study the possibility of a similar helper action of B lymphocytes in a syngeneic system, and also their possible participation in the mechanism of the PTL effect. The investigation described below was devoted to the study of this problem, and involved the study of the effect of B lymphocytes arising from different organs on the formation of hematopoietic colonies by stem cells, freed from PTL, mature lymphocytes, and cells adherent to plastic, as well as on interaction of stem cells and PTL during colony formation.

EXPERIMENTAL METHOD

The donors and recipients of the cells and their fractions were male (CBA × C57BL/6)F_1 mice weighing 18-20 g, obtained from the Stolbovaya nursery (Academy of Medical Sciences of the USSR) and the Central nursery (Academy of Sciences of the USSR). The bone marrow cells were flushed out of the femora of the mice with medium 199 and fractionated, by methods based on the panning method [7] and mass cytolysis [3]. The fractionation scheme shown in Fig. 1 was used for the bone marrow cells. During fractionation of spleen and lymph node cells (pooled inguinal, popliteal, and axillary lymph nodes) only cells carrying membrane immunoglobulins (Ig^+-cells) (fraction 1) were isolated.

Expression of surface markers was assessed by immunofluorescence (Thy-1,2, Ig) and cytotoxic (SC-1, MBLA) tests. Monoclonal antibodies to Thy-1 2 antigen (G4), immune sera to PTL SC-1 antigen (brain antiserum exhausted with liver and thymocytes), and immune rabbit serum to mouse MBLA antigen (provided by Professor N. A. Kraskina) were used. Affinity-purified IgG-antibodies to immunoglobulins of classes M and G (specificity verified by Ouchterlony's method), isolated from immune rabbit serum, also were used.

Colony formation in the spleen was assessed by the exotest in [8]. For this purpose recipient mice were irradiated in a dose of 8.5 Gy on a "Stebel'" ^{137}Cs γ-ray source The doses of cells injected corresponded to their yield during fraction-

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