served when the ratio of killer to target cells was 100:1 and 50:1, but as regards normal killer cells, a significant decrease in CTI was observed only when the ratio was 50:1 (cytotoxicity was determined 11 days after PHE in BALB/c mice). The results are in agreement with data in [1], according to which, on the 9th day after PHE on CBA mice activity of normal killer cells also was reduced, but they are contradicted by other data [4]. In the latter case, however, the stimulating effect of PHE on normal killer cell function occurred after removal of half of a lobe of the liver from the mice, and not two-thirds of the liver according to the usual method, and this might have affected the results. A decrease in cytotoxicity of T and normal killer cells after PHE may be one cause of the more rapid growth of tumors in animals with a regenerating liver [11, 12].

Depression of killer activity and of the graft versus host reaction after PHE is evidently due to the action of suppressor cells, as is shown by the example of lowering of the helper function in MLC. The authors are grateful to Professor B. B. Fuks for providing the reagents for the membrane-toxicity test.

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INTERLEUKIN 2 PRODUCTION IN PATIENTS WITH THYROID DISEASES

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KEY WORDS: interleukin 2; thyroid gland; autoimmune process.

The T-cell growth factor interleukin 2 (IL 2) plays a key role in maintaining the function and permitting expansion of various classes of cytotoxic lymphocytes [8-10]. During antigenic stimulation, intercellular interactions of complex structure and realized through the lymphokine cascade are activated; one essential component of this cascade is production of IL 2 by antigen-specific T helper cells [6, 12].

One of the factors controlling the level of IL 2 production may be the endocrine system. It has been shown that ACTH and glucocorticoids directly control IL 2 production [7, 11, 12]. Thyroid hormones possible play a similar role as well, and it was therefore decided to study the effect of differences in thyroid function on the IL 2 level, in states of hyper- and hypothyroidism. Mice of strains predisposed to the development of autoimmune diseases are known to have a low level of IL 2 production [4, 5]. In patients with rheumatoid arth-

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TABLE 1. IL 2 Activity in Thyroid Diseases

Nos. of Patients	Nature of disease	Thyroid function	SI for groups (M ± m)
		Cases of series I	
1-4	Control	Euthyroidism	66.5 ± 3.23
5-12	Autoimmune thyroiditis	Thyrotoxicosis (No. 5)	
		Hypothyroidism (No. 6)	73.0 ± 6.88
		Euthyroidism (No. 7-12)	
13-16	Carcinoma	Euthyroidism	72.6 ± 2.64
17-22	Diffuse toxic goiter	Compensated thyrotoxicosis (No. 17-19)	42.4 ± 2.48 P < 0.01
		Decompensated thyrotoxicosis (No. 20-22)	74.1 ± 3.88
25-30	Nodular goiter	Euthyroidism	75.4 ± 1.59 P < 0.001
		Cases of series II	
1-7	Control	Euthyroidism	10.3 ± 2.2
8-12	Autoimmune thyroiditis	Hypothyroidism (No. 8-10)	15.5 ± 2.66
		Euthyroidsm (No. 11-12)	14.8 ± 1.31
13-14	Diffuse toxic goiter	Compensated thyrotoxicosis	11.9 ± 4.8
15-17	Nodular goiter	Euthryoidism	11.3 ± 3.52
		Cases of series III	
1-4	Control	Euthyroidism	12.95 ± 2.38
5-10	Diffuse toxic goiter	Decompensated thyrotoxicosis (No. 5-8)	16.35 ± 0.32
		Compensated thyrotoxicosis (No. 9-10)	13.7 ± 1.0

ritis, production of this lymphokine also is depressed [3]. The possibility cannot be ruled out that in thyroid diseases whose pathogenesis is linked with an autoimmune process, IL 2 production also is disturbed.

EXPERIMENTAL METHODS

IL 2 activity was determined in 15 clinically healthy persons aged 20-50 years and in 42 patients with various thyroid diseases (aged 17-65 years). The patients were studied in the Department of Endocrinology by the usual clinical and laboratory methods. The state of thyroid function was assessed by radioimmunoassay with determination of total T_4 and T_3 and of pituitary thyrotrophic hormone. The final diagnosis was confirmed histologically after operative treatment of the patients. Some patients (the group with diffuse toxic goiter) were treated with methimazole (carbimazole).

Peripheral blood lymphocytes were isolated on a Ficoll–Hypaque density gradient (d = 1.077 g/ml). To obtain IL 2, lymphocytes (10^6/ml) were incubated with 5 μg/ml of concanavalin A (con A) in medium RPMI 1640 with 5% calf embryonic serum, glutamine (2 mM), and 2-mercaptoethanol ($5 \cdot 10^{-5}$ M) for 24 h in a CO_2 incubator. The cell-free supernatant of the cultures was used as the source of IL 2 after neutralization of the residual lectin with α-methyl-D-mannoside (20 mg/ml). IL-2 activity was assessed by its ability to maintain proliferation of T blast cells [2]. To obtain T blast cells, BALB/c mouse spleen cells ($5 \cdot 10^6$/ml) were cultured for 4 days in the presence of con A (5 μg/ml). The cells were washed twice before testing and transferred to fresh culture medium. Double dilutions

of IL 2 were prepared in flat-bottomed 96-well Linbro panels in a volume of 100 µl. An equal volume of the suspension of T blast cells (10^6/ml) was then added to the wells. After incubation for 14 h, ^{3}H-thymidine was added to the wells in a volume of 50 µl (0.5 µCi, specific radioactivity 24 Ci/mmole). Another 4 h later the cells were washed, the DNA precipitated with 5% TCA solution and retained on filters, and the radioactivity of the samples was estimated on a Packard Tricarb liquid scintillation counter. The stimulation index (SI) was determined by the following equation:

$$SI = \frac{\text{number of counts per minute in the presence of IL 2 (dilution 1:2)}}{\text{number of counts per minute without IL 2}}$$

RESULTS

The results of the three series of experiments are summarized in Table 1. In each series low variability of SI, reflecting IL 2 activity, was observed in the control group, and the changes in SI in all series of experiments were similar in direction in patients with a particular diagnosis, so that they could be considered together.

In the most numerous group of patients (with diffuse toxic goiter, 14 cases) IL 2 production depended on the stage of treatment of the disease. All patients were given methimazole in a daily dose of 20-40 mg. In patients in the early stage of conservative treatment, before permanent euthyroidism and had been achieved (the first 2 weeks of treatment) a high level of IL 2 production was discovered. The value of SI in patients with decompensated thyrotoxicosis in series I amounted to 111.4% of the control, and in series III to 127% of the control. In patients in the phase of euthryoidism during treatment with methimazole (under treatment for over 6-8 weeks) IL 2 production was lower than in the control group, namely 63.7% in series I, falling in individual patients to 57.4% (patient No. 19).

The state of decompensated thyrotoxicosis in patients with diffuse toxic goiter thus corresponds to increased functional activity of P lympocytes, responsible for IL 2 production, in agreement with the view that certain functions of the T cell system of immunity are modified in this disease [1]. Enhancement of con A-induced IL 2 production in these patients may also be connected with an increase in the fraction of IL 2 producers in the cell population tested. The thryostatic agent methimazole, when administered in the long term (over 4-6 weeks), leads to suppression of IL 2 production, in agreement with the view that this drug has an immunosuppressive action [1]. Lowering the level of IL 2 production may perhaps be associated primarily with a fall in the level of synthesis of thyroid hormones under the influence of methimazole. An even more marked suppressive effect was obtained in patient No. 13 in series II, receiving combined treatment with methimazole (10-30 mg daily) and prednisolone (10-60 mg daily) for 5 months. SI was 7.1 with IL 2 in a dilution of 1:2. These results are in agreement with data on the regulatory effect of glucocorticoid hormones and their synthetic analogs on production of monokines and lymphokines including IL 2 [7, 11, 12].

In patients with autoimmune thyroiditis (13 cases) in the euthyroid phase a tendency was found for the level of IL 2 production to rise with an increase in SI to 110.4% compared with the control in series I and to 143.4% in series II.

However, in individual patients of this group with disturbances of thyroid function, IL 2 production was lower than in the control.

In patients with nodular euthroid goiter (15 cases) IL 2 production also was increased. The size of the goiter in all patients corresponded to the II-III degree of enlargement, and the presence of the nodule and an increase in its size have been observed for 3-7 years. These patients had received no medical treatment before the investigation of their IL 2 production. A malignant thyroid tumor was diagnosed in four patients, a follicular carcinoma (T1NOMX) in three of them and a papillary carcinoma (T3N3MX) in one. Thyroid function in the patients of this group corresponded to euthyroidism. In patients with malignant nodular goiter IL 2 production was maintained relative to the control or exceeded it somewhat. These results suggest that in the case of slowly progressive growth and comparatively late metastisization, which is a feature of human thyroid neoplasms, IL 2 production may remain preserved or somewhat increased.

It can be concluded from the results as a whole that thyroid diseases are accompanied by changes in IL 2 production. The level of thyroid function and the character and duration of treatment are undoubtedly important factors. In particular, thyrostatic therapy

with the carbimazole derivative methimazole inhibits IL 2 production. These same factors (thyroid function, anatomical features of the gland, the phase of the disease, the character of treatment) evidently determine the level of IL 2 production in patients with autoimmune thyroiditis.

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INHIBITION OF HETEROPHIL ANTIBODIES TO MYOCARDIAL INTERSTITIAL CONNECTIVE TISSUE ANTIGENS BY α-D-GALACTOSE

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KEY WORDS: heterophil antibodies; myocardium; connective tissue; α-D-galactose.

In previous investigations the writer found heterophil antibodies, reacting with interstitial connective tissue (ICT) cells of bovine myocardium in sera from patients with rheumatic fever and other diseases of the heart and connective tissue. It was shown that the frequency of discovery and the titers of these antibodies were considerably higher in patients with active rheumatic fever than in patients with inactive rheumatic fever and healthy blood donors [2, 4]. The heterophil bovine antigen (HBA) is tissue-specific and is found in all animals in myocardial ICT, in other connective tissue cells of various bovine organs, and also on erythrocytes. It has been shown that reactions of antibodies with bovine myocardial ICT cells are inhibited by D-galactose [3].

Various human diseases are known to be accompanied by the appearance of autoantibodies or heterophil antibodies in the serum. It has been shown recently that the specificity of some of them is linked with certain carbohydrate sequences. These include agglutinins in hemolytic anemia, directed against I- and i-antigens [5], antibodies against T antigen (Thomsen–Fridenreich antigen [8]), whose antigenic specificy is linked with terminal β-D-galactose, heterophil antibodies of the Hanganutsiu–Deicher and Forssman type, etc. [6].

The aim of this investigation was to study the nature of the immunodominant HBA group by inhibiting the reaction of heterophil antibodies with bovine myocardial ICT by various mono- and disaccharides of known chemical structure. Antibodies to HBA also were compared with other antibodies against carbohydrate determinants.

EXPERIMENTAL METHODS

Altogether 23 sera were tested: 10 from patients with rheumatic fever in the active phase, eight from patients with rheumatic fever after artificial heart valve replacement

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