Blood Lymphocyte Proliferation Reaction in Autoimmune Thyropathies

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Spontaneous and stimulated reaction of lymphocyte proliferation and blood levels of CD25- and CD120-expressing lymphocytes were studied in patients with autoimmune thyroiditis and diffuse toxic goiter. Activation of CD25- and CD120-presenting function of blood lymphocytes was revealed. The findings indicate that the course of autoimmune thyroiditis and diffuse toxic goiter is associated with increased levels of basal, mitogen- and cytokine-stimulated proliferation of blood lymphocytes, particularly pronounced in thyroiditis in the euthyrosis stage.

Key Words: *lymphocyte proliferation reaction; autoimmune thyroiditis; diffuse toxic goiter*

Autoimmune thyropathies (AITP) autoimmune thyroiditis (AIT) and diffuse toxic goiter (DTG)) are among the most incident diseases in the structure of thyroid abnormalities. Despite numerous studies of this problem, the mechanism of pathogenetic reactions in AITP remains unknown [3,7].

Autoimmune thyropathies belong to a group of complex polygenic diseases, their development is determined by numerous genetic, endo- and exogenous factors provoking the antithyroid immune reaction manifesting in high production of autoantibodies to components of thyrocyte membrane and thyroid hormones (thyroglobulin, thyroperoxidase, thyrotropin receptor, *etc.*) [1,7,9]. In addition, imbalance between the pro- and antiinflammatory cytokines leads to changes in the immune homeostasis, primarily to changes in proliferative activity of immunocompetent cells [2,3]. Impairment of B and T lymphocyte proliferation processes is an important component in the progress of destructive and inflammatory processes in the thyroid tissue.

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We evaluated proliferative activity of peripheral blood lymphocytes in AITP.

MATERIALS AND METHODS

Sixty patients with AITP aged 19-55 years (mean age 42.1±3.9 years) were examined. The diagnosis was in each case verified with consideration for clinical picture and paraclinical parameters in accordance with routine criteria [1]. The patients were divided into 3 groups in accordance with the nosological entities and functional activity of the thyroid. Group 1 consisted of 20 patients with AIT in a state of euthyrosis (hormonal profiles within the norm); group 2 consisted of 16 patients with AIT with thyroid hypofunction (mean level of thyrotropin 10.59±3.25 mMI/liter), the concentration of free thyroxin corresponded to 6.49±1.44 nmol/liter). Mean duration of AIT (from the moment of diagnosis) was 6.0±2.7 years. Group 3 consisted of 10 patients with DTG (thyrotoxicosis of different severity, with thyrotropic hormone level decreased to 0.07±0.03 mMI/liter, while the level of free thyroxin increased to 54.67±10.41 nmol/liter and of antibodies to peroxidase reaching 349.47±89.00 U/liter.

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Patients with DTG were examined during thyrotoxicosis at the debut of the disease (mean duration of the disease 3-5 months) before prescription of thyrostatic therapy in order to exclude possible effects of antithyroid drugs on the studied parameters. Control group consisted of 14 normal subjects without thyroid abnormalities.

Lymphocytes isolated from the whole peripheral blood by gradient centrifugation were analyzed.

The lymphocyte proliferation reaction was evaluated by the MTT test after 72-h culturing of lymphocyte suspension in the absence and presence of stimulants: 10 μg/ml *Escherichia coli* 026:B6 LPS (Sigma), 30 μg/ml phytohemagglutinin (PHA; Becton Dickinson), 10 μg/ml recombinant IL-2 (Biosourse), or 2 μg/ml recombinant TNF-α (Biosourse). Optical density of cell suspensions was measured on an Uniplan analyzer (PICON) at λ=560 nm. Lymphocyte differentiation antigens were detected using CD25 monoclonal antibodies (Sorbent Company) in the lymphocytotoxic test and Monoclonal antihuman TNF R1-Fluorescein (R&D Systems Inc.) by immunofluorescent method.

The significance of differences in the samples not conforming to normal distribution was evaluated using Mann—Whitney nonparametric test, samplings with normal distribution were compared using Student's test for independent variables. The critical level of significance for verification of statistical hypotheses was 0.05 [5].

RESULTS

The levels of spontaneous and mitogen-stimulated lymphocyte proliferation in AITP patients significantly surpassed the control, irrespective of the disease form and thyroid function (Table 1). In group 1, the level of PHA-stimulated lymphocyte proliferation was 1.5 times higher than in the control ($p<0.001$) and in groups 2 ($p<0.001$) and 3 ($p<0.001$).

TABLE 1. Proliferative Activity and Content of $CD25^+$ and $CD120^+$ Peripheral Blood Lymphocytes in Donors and AITP Patients ($X \pm m$)

Parameter	Donors (n=14)	Group 1	Group 2	Group 3
Basal proliferation, opt. dens. units	0.28±0.01	0.36±0.02 $p_d<0.001$	0.33±0.01 $p_d<0.001$	0.35±0.02 $p_d<0.001$
Stimulated proliferation, opt. dens. units				
PHA	0.34±0.02 $p_b<0.01$	0.50±0.03 $p_d<0.001$ $p_b<0.001$	0.39±0.02 $p_d<0.001$ $p_b<0.001$ $p_1<0.001$	0.47±0.02 $p_d<0.001$ $p_b<0.01$ $p_1<0.01$
LPS	0.33±0.02 $p_b<0.01$	0.46±0.02 $p_d<0.001$ $p_b<0.01$	0.44±0.02 $p_d<0.001$ $p_b<0.001$	0.46±0.02 $p_d<0.001$ $p_b<0.01$
IL-2	0.36±0.02 $p_b<0.01$	0.78±0.04 $p_d<0.001$ $p_b<0.001$	0.62±0.03 $p_d<0.001$ $p_b<0.001$ $p_1<0.001$	0.70±0.04 $p_d<0.001$ $p_b<0.001$ $p_1<0.001$
TNF-α	0.43±0.02 $p_b<0.01$	0.94±0.05 $p_d<0.001$ $p_b<0.001$	0.74±0.04 $p_d<0.001$ $p_b<0.001$ $p_1<0.001$	0.77±0.04 $p_d<0.001$ $p_b<0.001$ $p_1<0.001$
$CD25^+$ cells, %	13.55±0.68	16.86±0.84 $p_d<0.01$	12.31±0.62 $p_1<0.05$	15.55±0.78 $p_d<0.01$
$CD120^+$ cells, %	6.25±0.31	11.32±0.57 $p_d<0.01$	9.30±0.47 $p_d<0.01$ $p_1<0.01$	11.78±0.59 $p_d<0.01$ $p_2<0.01$

Note. p_d: compared to donors; p_1: compared to group 1 patients; p_2: compared to group 2 patients; p_b: compared to basal level of proliferation.

The level of LPS-stimulated proliferation in patients with AIT (eu- and hypothyroid stages) and DTG was 1.3 times higher than in donors.

Lymphocyte proliferation in response to PHA stimulation reflects functional activity of T-cells, while the response to LPS stimulation indicates functional activity of B-cells. We detected high capacity of T- and B-cells to multiplication, which, no doubt, can be responsible for more severe course of the autoimmune process. In addition, mitogen-stimulated lymphocyte proliferation significantly surpassed basal proliferation, which was most demonstrative in group 1 patients in response to PHA stimulation ($p<0.001$; Table 1). This, in turn, indicates higher reactivity of T- compared to B-cell immunity in AIT.

Similar changes were observed in AITP: proliferation of lymphocytes stimulated by recombinant cytokines (Table 1) was higher than in donors, particularly (like in mitogen-stimulated cell reaction) in group 1 patients, in whom the level of lymphocyte proliferation stimulated by recombinant IL-2 and TNF-α virtually 2-fold surpassed the normal. Many authors claim that these cytokines most intensely modulate the proliferation of lymphocytes [4,6,8]. Increased serum levels of IL-2 and TNF-α were detected in AIT patients [2,3,7].

The most pronounced increase of proliferative activity in comparison with basal proliferation of lymphocytes was noted in response to stimulation by recombinant TNF-α. The highest level of TNF-α-stimulated cell proliferation was observed in group 1.

It is known that immunocompetent cells can be activated by autoantigen, which leads to fulminant increase in the production of IL-2 and TNF-α, increase in their concentrations stimulating the expression of the corresponding high-affinity receptors and triggering cascade reactions eventually causing an increase in proliferative activity of lymphocytes [3] responsible for destruction of follicular epithelial cell.

The percent of lymphocytes expressing CD25 (IL-2 receptor) increased by 1.2 times ($p<0.01$) in groups 1 and 3, while in group 2 patients this parameter was comparable to that in donors. The percent of lymphocytes expressing CD120 (TNF-α receptor) significantly surpassed the control in all groups. Maximum expression of these receptors on lymphocytes was noted in patients of groups 1 and 3 (Table 1).

Analysis showed that AIT (irrespective of the thyroid function) and DTG are associated with increased levels of basal, mitogen- and cytokine-stimulated proliferation of blood lymphocytes, the most pronounced in AIT during the euthyroid stage. The level of cytokine-stimulated proliferation was significantly (2-fold; $p<0.01$) higher than the level of mitogen-stimulated proliferation of lymphocytes, this proving the leading role of cytokine (particularly mediated by TNF-α) activation of proliferation of antigen-specific T and B lymphocytes forming clones to different antigenic thyrocyte epitopes. Activation of CD25 and CD120 presenting function of lymphocytes is the factor responsible for high level of blood lymphocyte proliferation in response to cytokine stimulation in AITP. This indicates that the pool of reactive lymphocytes exhibiting high susceptibility to growth factors (IL-2 and TNF-α) stimulating lymphocyte proliferation is permanently replenished during AITP.

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