Effect of the Synthetic Regulatory Peptide Thymohexin on the Production of Various Classes of Immunoglobulins

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Some mechanisms of the immunoregulatory effect produced by the new drug thymohexin (immunophan), which contains synthetic hexapeptide as a pharmacological agent, are studied. Administration of the drug in a culture of polyclonally activated mononuclear cells from healthy donors intensifies IgM, IgG, and IgA production and inhibits IgE synthesis. Treating intact mononuclear cells with thymohexin does not significantly affect the production of immunoglobulins. The drug is found to intensify IgA synthesis in cases of genetically determined insufficiency and to suppress the production of IgE by lymphocytes in patients with atopic dermatitis. The immunoregulatory effect of the drug is shown to be mediated via cell-cell interaction in the system of transfer of thymohexin-activated mononuclears.

Key Words: *thymohexin; mononuclears; immunoglobulins; regulatory cells; atopic dermatitis*

Recent achievements in the design of drugs for the correction of disorders in the immune system are mainly due to the discovery of thymic regulatory peptides. Based on individual representatives of these peptides, such second-generation drugs as thymosin α_1 and some others have been developed and are now being used clinically for the treatment of a number of disorders accompanied by alterations in T-cell immunity [7,9]. However, the second-generation drugs have not found wide application due to complications in synthesis technology as well as to the polyfunctional properties of thymus regulatory peptides.

Immunocorrective peptides of the third generation, containing synthetic short fragments of thymus regulatory peptides, are now being proposed [5]. Meanwhile, further search for active peptide fragments is exhausted due to the limited assortment of natural regulatory peptides of the central immune organs.

Obtaining modified synthetic regulatory peptides or peptide drugs of the fourth generation may be one of the possible ways of enlarging the arsenal of immunocorrectives, including those for the treatment of congenital and acquired immune disorders. To this end, a hexapeptide of original structure has been obtained as the pharmacological agent of a new fourth-generation drug, thymohexin (TH) (immunophan). Limited clinical trials have proved its positive effect on the production of specific antibodies in patients with chronic brucellosis as well as an achievement of seroconversion in patients with chronic viral hepatitis B [4,8].

A study of the effect of TH on the synthesis of different classes of immunoglobulins (Ig) by mononuclear cells (MNC) of healthy donors and patients with noninfectious pathology was undertaken to assess the mechanism of action of TH and the sphere of its use.

MATERIALS AND METHODS

MNC isolated in a Ficoll-Verographin density gradient from heparinized peripheral blood were obtained from healthy donors and from atopic der-

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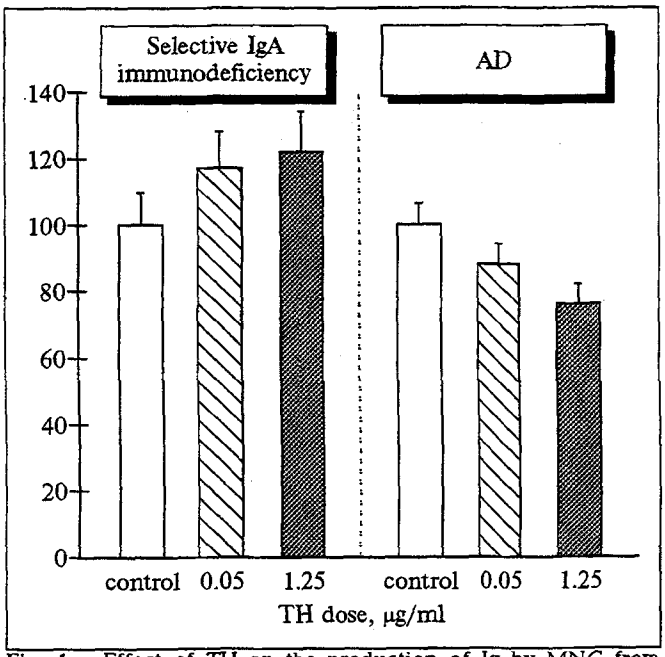

Fig. 1. Effect of TH on the production of Ig by MNC from patients with selective IgA immunodeficiency ($n=3$) and AD ($n=6$), in % as related to the control. Here and in Fig. 2: an asterisk denotes $p<0.05$ as compared to the control.

matitis (AD) and IgA selective immunodeficiency patients. Ig production was determined in supernatant of MNC cultured for 8 days in the presence of pokeweed mitogen (PM, 10 μg/ml) in MNC cultures and in RPMI-1640 medium with 10% ETS, 2 mM L-glutamine, 10 mM HEPES, 2×10^{-5} mercaptoethanol, and gentamicin in a humid atmosphere with 5% CO_2. MNC treated with TH in the corresponding concentration for 1 h in RPMI-1640 were then washed twice in the same medium and placed in complete culture medium.

IgA and IgE synthesis was studied in a spontaneous MNC culture or a culture treated with recombinant interleukin-4 (50 IU/ml, Biotekhnologiya) with incubation for 8 days in Iscove's medium with analogous additives.

TH-activated cells or regulatory cells (RC) were obtained by pulsed action of the preparation (for 1 h) on fresh cultures of MNC with subsequent two-fold washing free of preparation and then added to an autologous culture of mononuclears of target cells (TC) at 2.5%.

The level of Ig synthesis was determined by the avidin-biotin immunoenzyme sandwich method. Polyclonal anti-human Ig antibodies immobilized in the solid phase were used as the first antibodies. The second were biotinilated monoclonal antibodies specific against heavy chains of human Ig. The complex was visualized using streptavidin-polymeric horseradish peroxidase conjugate. The optical density was measured at 492 nm. Calibration curves were obtained using affinity-purified human Ig of corresponding classes. The Ig amount in the studied samples was expressed in weight units per milliliter of volume.

The results were processed statistically using the Student t test at a significance level no less than $p<0.05$.

RESULTS

The results of the study of the effect of TH on the PM-induced production of Ig in different classes of MNC from healthy donors are listed in Table 1. The findings attest that the addition of TH to human lymphocyte cultures results in a sharp stepping-up of M, G, and A Ig synthesis. Conversely, IgE production did not change significantly for TH doses of 0.5 and 5.0 μg/ml, while at 0.05 μg/ml a marked suppression of the IgE response was noted. Thus, the data demonstrate a diverse effect of TH on the production of IgE as well as of M, G, and A Ig.

It should be noted that these data were obtained using a model of induced Ig synthesis. Treating intact cells from healthy donors with TH does not cause marked changes in the production of the main Ig classes in MNC culture without the addition of PM. This indicates that the regulatory peptide does not have any marked effect on spontaneous Ig production by MNC from healthy donors. Therefore, it was of interest to study the effect of the preparation in pathological states with impaired Ig production. For this, MNC from IgA selective immunodeficiency patients and cells from AD patients with elevated IgE production were used.

The experimental data in Fig. 1 testify that TH stimulates IgA production in congenital insufficiency and inhibits IgE synthesis in the case of hyperproduction in AD patients. Thus, it may be assumed that TH regulates IgA and IgE production depending on the level of their synthesis.

The ambiguous effect of TH on IgM, IgG, and IgA production as well as on IgE synthesis may be important for its expanded clinical use in patients with humoral immunity disorders in infectious and noninfectious pathology. For instance, the use of TH in patients with chronic brucellosis increases the titers of specific IgM-, IgG-, and IgA-antibodies while IgE-antibody titers change relatively less, this being considered as a beneficial factor of the therapy, which eliminates complications caused by an unbalanced rise of reacting IgE-antibodies [4].

This feature of the influence of the fourth-generation peptide preparation on the production of

certain Ig classes may be brought to bear by changing the production of immunity transmitters and the mechanisms of cell-cell reactions. Since IL-4 is one of the key transmitters controlling IgE synthesis [6], the effect of TH on human IgE production by MNC was studied in the presence of this cytokine. In view of the diverse action of the preparation on IgA and IgE synthesis, a comparative analysis of the two Ig classes seemed to be desirable.

TH at 0.01 μg/ml against the background of IL-4-dependent IgE synthesis causes its reliable suppression from 687 pg/ml (in the control) to 328 pg/ml (in the presence of TH) for simultaneous stimulation of IgA production. Since the suppressive effect of the preparation on IgE production in MNC cultures manifests itself both with and without IL-4, the inhibitory effect of TH on the production of IgE in MNC cultures is possibly realized without the involvement of IL-4.

This finding is in agreement with the observations of the influence of TH on IgA production in MNC cultures treated with IL-4. TH-activated MNC preserve the ability to intensify IgA production for the subsequent addition of IL-4 (from 1411 ng/ml in the control to 2321 ng/ml for the action of the preparation; $p<0.05$), just like cells not treated with IL-4, namely MNC from patients with congenital immunodeficiency of IgA and AD, and cells from healthy donors induced by PM, a polyclonal activator of B lymphocytes. These results show that treatment of MNC with IL-4 does not cause changes in the direction of the effect of TH on stimulation of IgA production or suppression of the IgE response.

The regulation of Ig synthesis is a multicomponent system which includes T- and B-lymphocyte interaction. This interaction is mediated both via soluble cellular products and via direct physical contact due to the corresponding membrane structures [2].

The role of cell-cell interaction in the realization of the effect of the regulatory peptide ion IgE and IgA production was assessed using the experimental model proposed earlier for the study of other biologically active substances [1,3]. MNC were treated with TH and then placed in an autologous culture of cells treated with IL-4 (TC). MNC with untreated TH cells added served as the control.

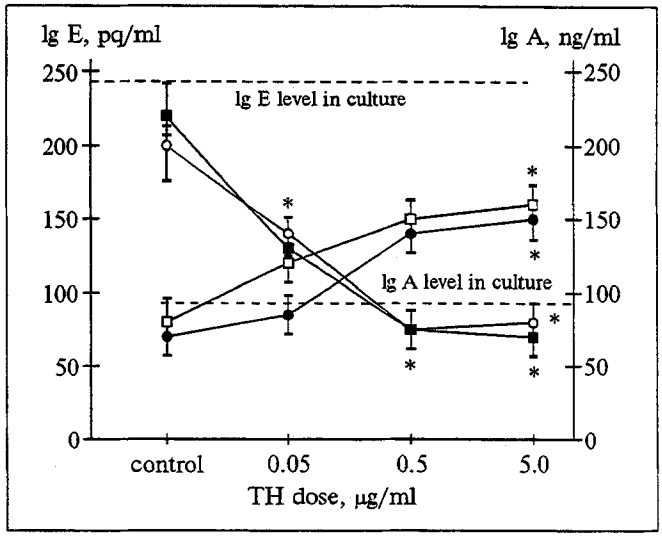

Fig. 2. Regulation of spontaneous IgE (*1,2*) and IgA (*3,4*) production in human MNC culture by TH-treated lymphocytes. *1, 3*) 2.5%; *2, 4*) 10% of cells.

The IgE level in the culture for the addition of 10% TH (RC)-treated cells to TC was 740, 440, and 390 pg/ml when TH was used at 0.05, 0.5, and 5 μg/ml, respectively. Control IgE production comprised 1300 pg/ml. The influence of 2.5% RC on IgE production was also characterized by a decrease of the IgE concentration from 1245 pg/ml in the control to 308, 800, and 435 pg/ml in TH in doses of 0.05, 0.5, and 5 μg/ml, respectively. The data are statistically reliable according to the Student *t* test.

Thus, RC interacting with TC free of the preparation cause a suppression of IgE production just like that with the direct action of TH. The findings attest that the immunosuppressive action of the regulatory peptide on IgE production is predominantly realized via the mechanism of cell-cell interaction and is preserved under conditions of excess IL-4.

TABLE 1. TH Influence on the Ig Production in PM-Induced Human MNC Culture ($M \pm m$)

TH dose, μg/ml	Ig concentration, ng/ml			
	IgM	IgG	IgA	IgE
Control	41±1.4	129±16	83±5.4	1.8±0.2
0.05	1481±22.8*	657±62*	1365±183*	1.0±0.2*
0.5	795±29*	672±57*	936±46*	2.0±0.1
5.0	781±40*	521±29*	657±57*	1.4±0.1

Note. An asterisk denotes $p<0.05$ as compared to the control.

In view of the diverse action of the regulatory peptide on the production of various Ig classes, we decided to examine the role of this interaction in realizing both the immunosuppressive and immunostimulatory effects of the preparation. For this, IgA and IgE *in vitro* production was studied when TH-activated cells were transferred to a culture of autologous MNC.

The results, presented in Fig. 2, demonstrate the immunosuppressive action of RC on IgE production as well as their immunostimulatory effect on IgA synthesis. The effect of RC manifests itself for their activation in a wide range of doses (0.5-5.0 μg/ml). Therefore, both effects, namely, IgA stimulation and IgE suppression, produced by the fourth-generation peptide preparation are realized via the mechanism of cell-cell interaction between RC activated by the preparation with TC.

It may be concluded that TH is a fourth-generation immunoregulatory preparation which intensifies IgM, IgG, and IgA production and suppresses IgE synthesis when MNC are polyclonally activated by PM. In the case of a pathological change of Ig synthesis due to congenital immunodeficiency of IgA or AD with IgE hyperproduction, TH exerts an immunocorrective effect on the production of both Ig classes. The mechanism of the immunoregulatory influence of TH on IgA and IgE synthesis is related to the interaction of immunocompetent cells and is realized independently of IL-4 addition.

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