BIOGERONTOLOGY

Expression of Argyrophilic Proteins in the Nucleolar Organizer Regions of Human Thymocytes and Thymic Epitheliocytes under Conditions of Coculturing with Vilon and Epithalon Peptides

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Vilon stimulated and Epithalon suppressed the expression of argyrophilic proteins in nucleolar organizer regions of thymocytes and epithelial cells, stimulating or reducing, respectively, the formation, assembly, and transport of ribosomes into the cytoplasm and thus determining the intensity of protein synthesis in these cells. A direct mitogenic effect of Vilon was also revealed: this peptide promoted thymocyte transformation into proliferating blast cells.

Key Words: *thymus; pineal gland; Vilon; Epithalon; argyrophilic proteins of nucleolar organizer region*

An important role in the maintenance of structural and functional homeostasis of cell populations is played by peptide regulators possessing autocrine, paracrine, and distant effects. They were isolated from all human organs and tissues, including the thymus (central organ of the immune system) and the pineal gland involved in the regulation of biorhythms [1,3,7,8]. Regulatory peptides thymopoietin, thymosin, thymalin possessing thymomimetic properties and regulating antigen-independent stage of T lymphocyte differentiation in the thymus and differentiation of peripheral immunocompetent T cells were isolated from the thymus. Regulatory peptide epithalamin possessing numerous functional activities was isolated from the pineal gland.

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Synthetic analogs of regulatory peptides were created on the basis of the data of comprehensive analysis of their structure. Vilon is a synthetic analog of thymalin and Epithalon is an analog of epithalamin [3,6], but the mechanisms of their effects on cells remain poorly understood. The effects of these peptides on ribosome formation attracted our interest; this process is regulated, among other mechanisms, by argyrophilic proteins of the nucleolar organizer regions (NOR).

We studied the effects of synthetic peptides Vilon and Epithalon on the expression of NOR argyrophilic proteins in thymocytes and epithelial cells during coculturing of these cells.

MATERIALS AND METHODS

The study was carried out on cocultured thymocytes and thymic epithelial cells from human embryos (aborted material at week 20 of gestation). The effects

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of three Vilon doses were studied: 2, 20, and 200 ng/ml. The preparation was added to the culture medium on day 2 and the study was carried out after 24 h. Argyrophilic proteins of NOR in cells were detected using silver nitrate. The duration of incubation needed for argyrophilic protein detection was 15 min for thymocytes and 10 min for epithelial cells (with consideration for different expression of argyrophilic proteins of NOR in thymocytes and thymic epithelial cells). The intensity of expression of argyrophilic protein was evaluated by the number of silver granules forming as a result of reaction. The granules were counted in 100 cells at ×1800. The mean number of silver granules per thymocyte (epithelial cell) nucleus was estimated and the mean number of granules per group was determined. The data were statistically processed using Student's *t* test.

RESULTS

Thymocytes formed a homogeneous cell population and were characterized by small size and nuclei with dense chromatin structure. The content of silver granules in thymocytes after 15-min incubation was not high (Table 1; Fig. 1, *a*). After 10-min incubation, there was virtually no reaction in thymocytes, while the reaction in epitheliocytes was pronounced (Table 1).

Addition of Vilon (2 ng/ml) to the culture medium induced appreciable changes in the thymocyte composition (Table 1). Increasing Vilon concentration in the medium to 20 and 200 ng/ml led to an increase in the number of silver granules in thymocytes. On the other hand, there was virtually no difference between the content of silver granules at Vilon concentrations of 20 and 200 ng/ml (Table 1). Notable morphological heterogeneity of the thymocyte population was observed after addition of 20 and 200 ng/ml Vilon. The number of large cells with fine chromatin structure morphologically similar to blast-transformed lymphocytes formed in response to antigen and mitogen stimulation increased. The content of silver granules was higher in blast-transformed cells than in thymocytes, reaching 5-7 and more granules in some cells (Fig. 1, *b*).

In epithelial cells the content of silver granules increased after treatment with Vilon in all concentrations. Similarly as in thymocytes, these changes were more pronounced after addition of 20 and 200 ng/ml Vilon (Table 1).

Epithalon suppressed the expression of argyrophilic proteins in thymocytes. The effect of the preparation did not depend on its concentration (Table 2; Fig. 1, *c*).

The number of silver granules in epithelial cells decreased in the presence of Epithalon in comparison with the control and was the same at all concentrations of the peptide (Table 2, Fig. 1, *c*).

Hence, peptide bioregulator Vilon stimulates the expression of NOR argyrophilic proteins responsible for the synthesis, assembly, and transport of ribosomes into the cytoplasm in thymocytes and epithelial cells, thus determining the intensity of protein production realized in these structures. Epithalon decreased the expression of argyrophilic proteins exerting an opposite effect on the ribosome formation processes and hence, on the intensity of protein production in these cells. Addition of Vilon into the culture medium stimulated the formation of large blastic cells among thymocytes. This transformation of lymphocytes into blastic proliferating cells, exhibiting the signs of increased metabolic activity, is observed after their stimulation with mitogens. One of the earliest processes observed under conditions of mitogen stimulation is an increase in functional activity of NOR and ribosome genes located in it, which is paralleled by an increase of the ribosomal RNA synthesis rate [9,11, 13,14].

Presumably, increased expression of argyrophilic proteins in cells and thymocyte blast transformation reaction observed after addition of Vilon into the culture medium result from the direct mitogenic effect of Vilon and reflect increased transcription activity of the ribosome genes, which can indicate the involvement of Vilon in mechanisms regulating the function of NOR and ribosomal genes. High expression of argyro-

TABLE 1. Content of Silver Granules in Thymocyte and Thymic Epitheliocyte Nuclei under Conditions of Their Coculturing in the Presence of Different Concentrations of Vilon in (n=100; $M \pm m$)

Parameter	Thymocyte nuclei	Thymic epitheliocyte nuclei
Control	3.15±0.08	22.07±0.47
2 ng/ml	3.30±0.13***	24.90±0.41**
20 ng/ml	3.73±0.12*	27.80±0.91**
200 ng/ml	3.93±0.10	28.80±0.59

Note. *p<0.001, **p<0.01, ***p<0.05 compared to the control.

TABLE 2. Content of Silver Granules in Thymocyte and Thymic Epitheliocyte Nuclei under Conditions of Their Coculturing with Epithalon in Different Concentrations (n=100; $M \pm m$)

Parameter	Thymocyte nuclei	Thymic epitheliocyte nuclei
Control	3.15±0.08	22.07±0.47
2 ng/ml	2.42±0.09*	20.50±0.51*
20 ng/ml	2.52±0.09*	20.70±0.51**
200 ng/ml	2.47±0.13*	20.90±0.56***

Note. *p<0.001, **p<0.01, ***p<0.02 compared to the control.

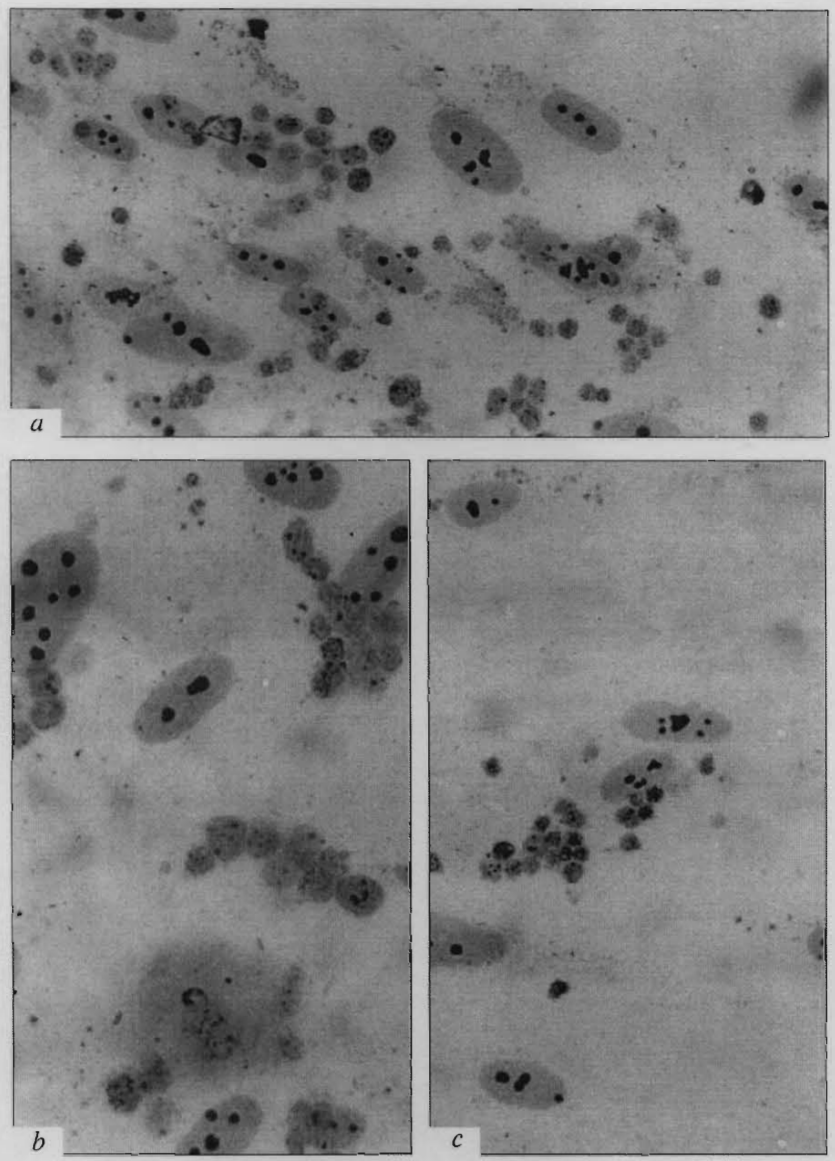

Fig. 1. Argyrophilic proteins of the nucleolar organizer regions in thymocytes and thymic epithelial cells under conditions of their coculturing. Reaction with silver nitrate. Incubation 15 min. *a*) control. Cell population consists of thymocytes and epithelial cells. Thymocytes are small, with few silver granules in the nuclei. Epithelial cells are large. Silver granules in the nuclei fuse into lumps, 2-4 per nucleus, ×720. *b*) exposure with Vilon in a concentration of 200 ng/ml. Number of silver granules in thymocytes is increased. Along with thymocytes of normal size, there are larger blast-transformed cells with high content of silver granules in the nuclei as well. In epithelial cell nuclei the content of argyrophilic material is increased. The number of silver lumps in the nuclei of some epithelial cells reaches 5-8, ×900. *c*) exposure with Epithalon in a concentration of 200 ng/ml. Decreased content of argyrophilic material in the nuclei of thymocytes and epithelial cells, ×720.

philic proteins in thymocytes and epithelial cells after addition of Vilon to the culture medium can also indicate stimulation of the cellular proliferative activity, which is in line with published data on the direct relationship between the proliferative activity of cells and the intensity of argyrophilic protein expression [2,4, 5,15]. Positive correlation between the content of argyrophilic proteins, cell growth fraction, and rate of cell proliferation is characteristic of the population of proliferating cells [10,12,15].

In contrast to Vilon, Epithalon suppressed argyrophilic protein expression in thymocytes and less so in epithelial cells, which probably reflects suppressed transcription activity of ribosomal genes and suppressed proliferative activity of cells. Moreover, decreased expression of argyrophilic proteins in thymocytes after addition of Epithalon to the culture medium can indicate the appearance of a population of better differentiated slowly proliferating cells.

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