

Immunomodulating Effects of Vilon and Its Analogue in the Culture of Human and Animal Thymus Cells

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We studied molecular mechanisms of immunoprotective effects of two dipeptides, AB-O and R-1, on cultured human and rat thymic cells. Both dipeptides were shown to increase the expression of lymphocyte differentiation marker CD5 in thymic cells. Dipeptide AB-0 induced T-cells precursor differentiation towards CD4⁺ T-helpers and its effect was weaker than that of dipeptide R-1. Dipeptide R-1 stimulates differentiation of CD5⁺ cells to mature T-helpers and cytotoxic CD8⁺ T cells and hence can be considered as a bioactive substance possessing immunomodulator and antiallergic activity.

Key Words: *dipeptides; cell culture; thymus; T cells*

The thymus is a central organ of the immune system and plays a leading role in the formation of T-cell immunity. Functional activity of the thymus in humans drastically decreases after puberty, which is associated with early involution of this organ [10]. Administration of short peptides for thymus function recovery is a new trend in immunology and pharmacology. It was found that di- and tripeptides are effective in age-related and radiation induced fading of the thymus function, immunopathological states caused by intensive physical exercise in athletes, and in autoimmune and tumor diseases [1,2,4,6]. Analysis of the molecular mechanisms underlying the effect of short immunomodulating peptides is now an important trend in molecular biology allowing identification of the targets of these bioactive substances and indications for their application. It has been demonstrated that short peptides induced differentiation, inhibited apoptosis, and stimulated proliferation of immune cells [5,7,8].

Here we compared the effects of dipeptides on the expression of lymphocyte markers in cultured human and rat thymic cells.

MATERIALS AND METHODS

Primary cultures of thymic cells from 3-month-old Wistar rats and human embryos were isolated at the Laboratory of Immunology of Aging, St. Petersburg Institute of Bioregulation and Gerontology. The specimens of embryonic thymus (gestation weeks 16-24) were obtained from D. O. Ott Institute of Obstetrics and Gynecology, Russian Academy of Medical Sciences, St. Petersburg. The cells were cultured in a medium containing 15% fetal calf serum, 82.5% RPMI, 1.5% HEPES buffer with L-glutamine on 3.5-cm Petri dishes pretreated with gelatin (Biolot) in a CO₂-incubator under standard conditions (5% CO₂, 37°C); trypsin-versen solution was used for subculturing (3:1). The cultures were grown in the presence of 0.9% NaCl (group 1), 0.05 ng/ml AB-0 dipeptide (group 2), or 0.05 ng/ml R-1 (group 3). Dipeptide AB-0 was chosen as the reference preparation (its immunomodulating effect is well studied). Previous studies have

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shown that dipeptide AB-0 regulates expression of some genes, stimulates thymocyte activation in culture (expression of HLA-DR and CD54), normalizes lymphocyte blast-transformation (LBT) response, and 2-fold reduces the incidence of respiratory infection in elderly individuals [1,3,9]. Dipeptide R-1 is a new bioactive substance promoting proliferation of thymic and spleen tissues in organotypic culture from young and old rats.

The peptide was added to the culture after each medium replacement. The cells were subcultured in 3 days after attaining confluence. Passage 3 cells were seeded to 24-well plate (Biolot) and immunocytochemical staining was carried out.

For immunocytochemical study, primary monoclonal antibodies to CD4 (T-helpers), CD5 (T and B cell precursors), and CD8 (cytotoxic T cells) diluted 1:50 and second biotinylated antimouse antibodies were used (all reagents were from Novocastra). Permeabilization was performed with 0.1% Triton X-100. The reaction was visualized with horseradish peroxidase and diaminobenzidine (EnVision Detection System, Peroxidase/DAB, Rabbit, Mouse). The results of immunocytochemical analysis were evaluated morphometrically using a computer-assisted microscopic image analysis system consisting of Nikon Eclipse

E400 microscope, Nikon DXM1200 digital camera, and Videotest-Morphology 5.2 software. In each case, at least 5 fields of view were analyzed at $\times 200$. The expression area was calculated as the ratio of the area occupied by immunopositive cells to the total area of cells in the field of view and expressed in percents. This parameter characterizes the number of cells expressing the studied transmembrane glycoprotein.

The data were processed statistically using Statistica 7.0 software. The significance of between-group differences was analyzed using Mann—Whitney *U* test, the most precise method for comparison of samples including 10-15 elements. The differences were significant at $p < 0.05$.

RESULTS

The expression of the studied glycoproteins CD4, CD5, and CD8 was verified in all control and experimental cultures of rat and human thymic cells.

Dipeptide R1 significantly increased the area of CD4 expression in the culture of rat thymic cells by 35% in comparison with the control, whereas dipeptide AB-0 produced no such effect (Table 1). In the culture of human embryo thymic cells, dipeptides AB-0 and R-1 increased the area of CD4 expression

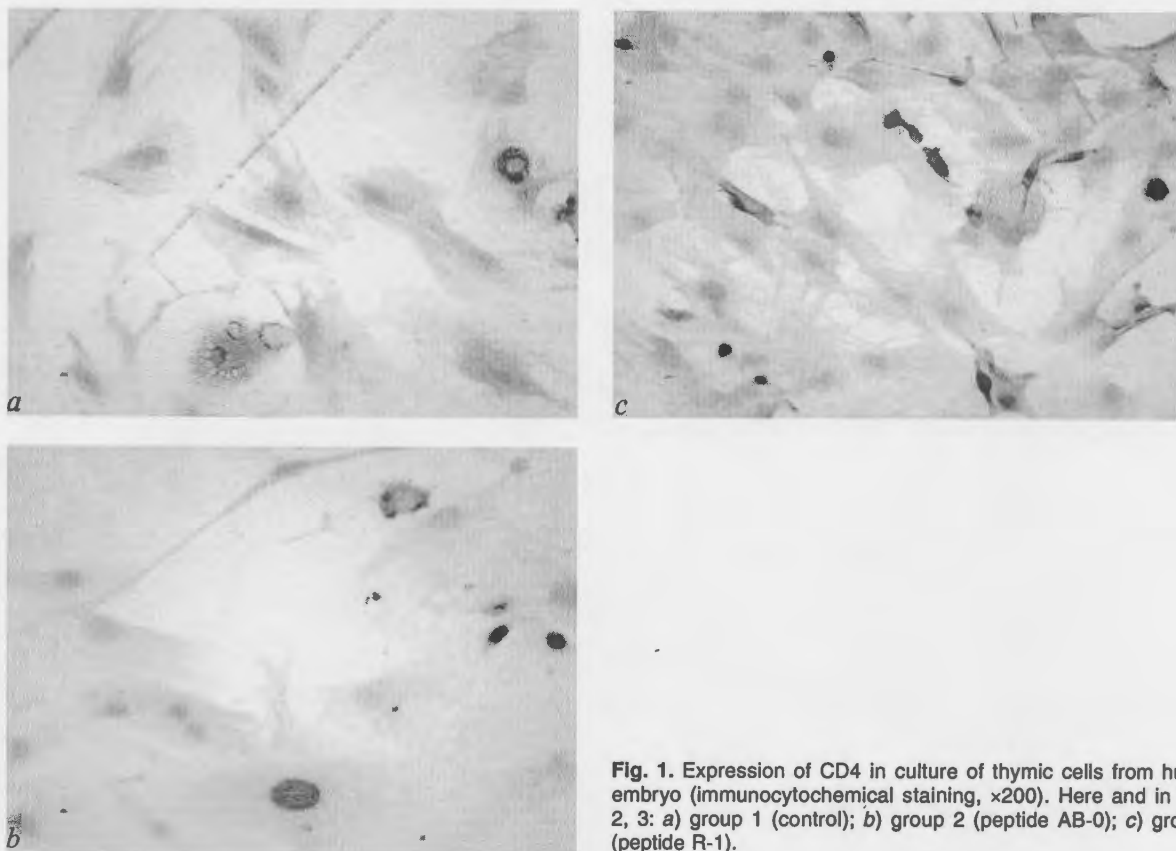


Fig. 1. Expression of CD4 in culture of thymic cells from human embryo (immunocytochemical staining, $\times 200$). Here and in Figs. 2, 3: a) group 1 (control); b) group 2 (peptide AB-0); c) group 3 (peptide R-1).

TABLE 1. Effect of Dipeptides on Expression of Immunocompetent Cell Markers in Thymic Cell Cultures

Group	Expression area, %					
	rat thymic cells			thymic cells from human embryo		
	CD4	CD8	CD5	CD4	CD8	CD5
Group 1 (control)	2.62±0.38	1.96±0.31	2.05±0.31	2.36±0.24	2.24±0.35	1.94±0.24
Group 2	3.17±0.51	2.26±0.24	3.64±0.51*	3.10±0.31*	2.86±0.28	2.82±0.37*
Group 3	3.54±0.47*	3.12±0.41*	2.78±0.47	3.68±0.56*	2.68±0.61	3.46±0.49*

Note. * $p < 0.05$ in comparison with the control.

by 31 and 56% in comparison with the control (Table 1; Fig. 1, *a-c*). Thus, the stimulating effect of dipeptide R-1 on T-helpers in rat and human thymus was more pronounced than that of dipeptide AB-0.

Peptide R-1 enhanced CD8 expression in the culture of rat thymic cells by 59% in comparison with the control, but did not change this parameter in the culture of human embryonic cells (Table 1; Fig. 2, *a-c*).

Of all studied markers, the expression of CD5 was most influenced by the test dipeptides, which attested to their effects on differentiation of thymic

cells. Under the effect of dipeptide AB-0, the area of CD5 expression in the culture of rat and human embryo thymic cells increased by 78 and 45%, respectively, in comparison with the control (Table 1; Fig. 3, *a, b*). Dipeptide R-1 stimulated CD5 expression by 78% only in human embryonic cells (Table 1; Fig. 3, *a, c*).

These findings suggest that lymphocyte markers CD4 and CD5, but not CD8 are the targets of dipeptide AB-0. Thus, this dipeptide probably stimulates differentiation of thymic T cell towards T helpers, which

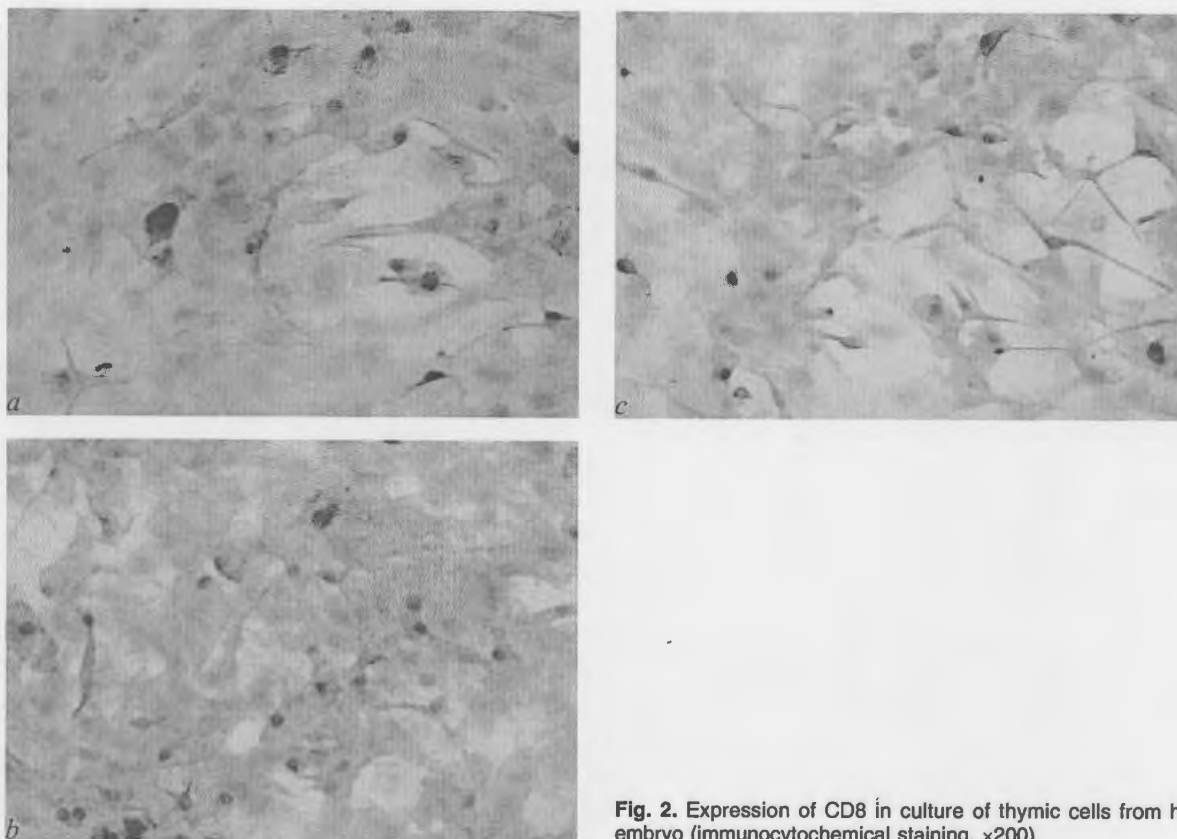


Fig. 2. Expression of CD8 in culture of thymic cells from human embryo (immunocytochemical staining, $\times 200$).

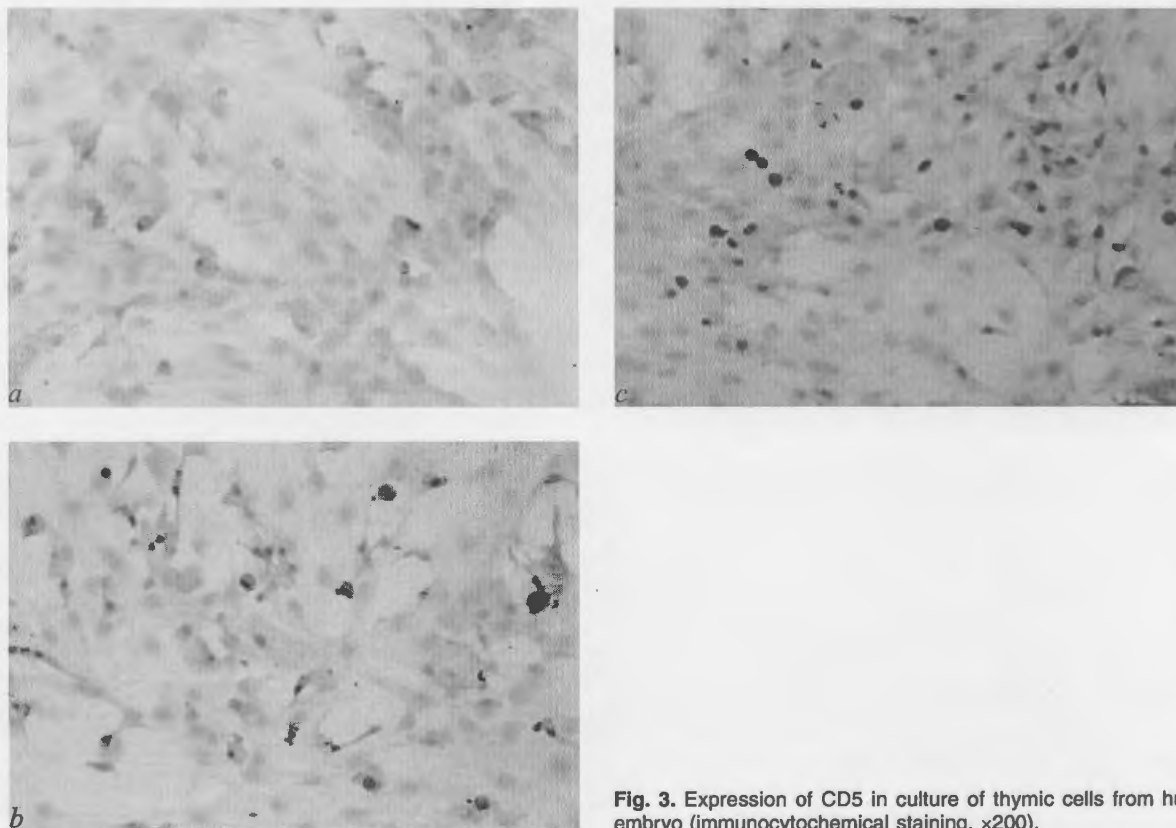


Fig. 3. Expression of CD5 in culture of thymic cells from human embryo (immunocytochemical staining, $\times 200$).

confirms its clinical immunostimulating and oncostatic effects in elderly patients [1,6]. Dipeptide R-1 produces the same, but more pronounced effects. Moreover, it induces differentiation of cytotoxic T cells, which is seen from enhanced expression of CD5 and CD8. It is known that reduced population of CD8⁺ cells is a cause of autoimmune diseases, while synthetic dipeptides thymogen and thymodepressin are effective preparation for the treatment of this pathology [2]. Further study of immunoprotective properties of peptide R-1 will help to characterize it more completely as a preparation promoting functional recovery of the immune system in immunodeficient and autoimmune diseases.

Thus, dipeptides AB-0 and R-1 exhibit pronounced immunomodulatory properties in cultures of human and rat thymic cells and stimulate lymphocyte differentiation; however, the effect of dipeptide AB-0 is directed towards T-helpers, while dipeptide R-1 stimulates differentiation of precursor cells towards cytotoxic T cells.

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