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Effects of Short Peptides on Thymocyte Blast Transformation and Signal Transduction along the Sphingomyelin Pathway

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Immunomodulating effects of synthetic peptides Vilon (Lys-Glu), Epithalon (Ala-Glu-Asp-Gly), and Cortagen (Ala-Glu-Asp-Pro) and possible involvement of the sphingomyelin signal transduction pathway in their effects in mouse thymocytes were studied. Vilon produced the most potent comitogenic effect on thymocyte proliferation and modulated comitogenic activity of interleukin-1β. Epithalon was less potent, while Cortagen produced no such effects. Vilon produced a more pronounced stimulatory effect on sphingomyelinase activity in mouse thymocyte membranes compared to Epithalon and Cortagen.

Key Words: *peptides; Vilon; Epithalon; Cortagen; interleukin-1; sphingomyelin pathway; blast transformation*

Aging and various pathologies are largely determined by activity of defense systems of the organism and their endogenous regulation. Targeted modulation of these mechanisms opens new vistas in the prevention of accelerated aging and treatment of age-related diseases. Of particular interest are physiological effects of natural and synthetic peptides modulating the effects of endogenous bioregulators and defense functions of the organisms.

Molecular mechanisms of cytokine interactions, specifically interactions of interleukin-1 (IL-1), the key endogenous regulator of defense reactions, with target cells and subsequent transduction of their signal play a key role in mobilization and modulation of congenital and acquired immunity [2,3]. Apart from adenylate cyclase and phosphoinositol pathways, the sphingomyelin pathway plays an important role in signal transduction in immunocompetent cells. This pathway triggered by hydrolysis of membrane sphingomyelin to second messenger ceramide under the effect of neutral sphingomyelinase (the key enzyme of sphingomyelin pathway) [3,4,7-10] transduces signals from cytokines IL-1β, tumor necrosis factor-α, and interferon-γ to immunocompetent cells. Modulating effects of other regulatory peptides (in particular, short peptides) on signal transduction pathway was not studied.

It is interesting to combine the study of the effects of short peptides in a new experimental model of the sphingomyelin pathway with evaluation of their effects on proliferative activity of thymocytes, in particular, on thymocyte proliferation modulated by IL-1. Comitogenic effect of IL-1β on proliferation of mouse thymocytes stimulated with lectin in a suboptimal dose is a key property of this unique regulator of defense reactions [2,12].

Here we studied possible direct mitogenic and comitogenic effects of short peptides on blast transformation of mouse thymocytes, their modulating effects on comitogenic action of IL-1β, and possible

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involvement of the sphingomyelin pathway in the transduction of their signals in immunocompetent cells.

MATERIALS AND METHODS

Experiments were carried out on 8-10-week-old male (CBA×C57Bl/6)F_1 mice (18-20 g). Short peptides were synthesized at St. Petersburg Institute of Bioregulation and Gerontology by targeted chemical synthesis based on the analysis of amino acid composition of complex preparations of the thymus (thymalin) — Vilon (Lys-Glu), epiphysis (epithalamine) — Epithalon (Ala-Glu-Asp-Gly), and brain cortex — Cortagen (Ala-Glu-Asp-Pro).

Lymphocyte activation and/or modulation of comitogenic effect of IL-1β was evaluated by the effect of test peptides on proliferation of mouse thymocytes stimulated with concanavalin A (ConA) in a suboptimal dose of 0.625 μg/ml and recombinant IL-1β (rIL-1β) in a concentration of 250 ng/ml [12]. Thymocytes were cultured in RPMI-1640 (Sigma) supplemented with 10% fetal calf serum (Sigma). Vilon, Epithalon, and Cortagen were used in concentrations from 0.25 to 0.25×10^{-12} ng/ml. Thymocyte cultured with mitogens (ConA or ConA+IL-1β) without peptides served as the control. The cells were cultured in a CO_2 incubator at 37°C, 5% CO_2, and 100% humidity. Incorporation of ^{3}H-thymidine (GIPKh) in dividing cells was counted on a scintillation β-counter (Beckman).

Membrane fraction from mouse thymocytes was obtained by modified method for isolation of somatic membranes from cultured cells [8]. Specific activity of neutral sphingomyelinase in thymocyte membrane fraction was measured as described previously [12] with modifications using ^{14}C-sphingomyelinase (Amersham) as the substrate. The effects of Vilon, Epithalon, and Cortagen on activity of neutral sphingomyelinase in thymocyte membranes were studied after preincubation of membrane fraction with test peptides in concentrations 0.1, 1, 10, 50, and 100 ng/ml in a water bath at 37°C for 30 min. In control samples test peptides were replaced with Tris-HCl buffer (50 mM, pH 7.4).

The results were statistically processed using Student *t* test.

RESULTS

None of the studied peptides produced a direct mitogenic effect: they did not induce thymocyte blast transformation in the absence of lectin or rIL-1β. However, Vilon (0.25×10^{-3}-0.25×10^{-5} and 0.25×10^{-9}-0.25×10^{-12} μg/ml) added to the thymocyte suspension containing ConA in an suboptimal dose significantly stimulated label incorporation in dividing cells, which proved its comitogenic effect on thymocyte proliferation (Fig. 1).

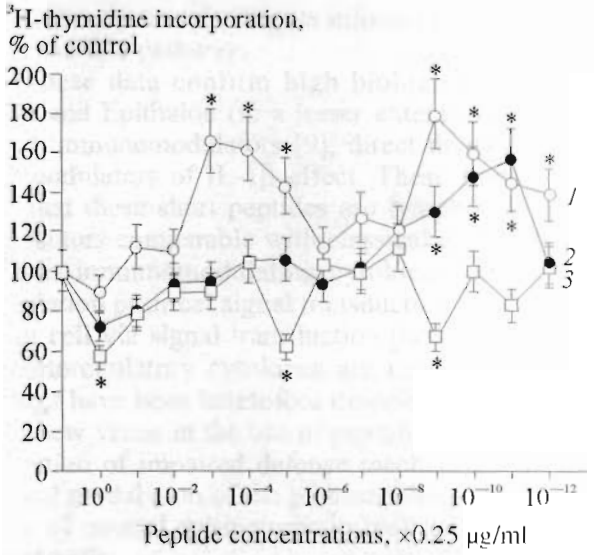

Fig. 1. Comitogenic effects of Vilon (*1*), Epithalon (*2*), and Cortagen (*3*) on proliferation of mouse thymocytes stimulated with concanavalin A in suboptimal dose (0.625 μg/ml). *$p<0.05$ compared to the control (concanavalin A without peptides).

Epithalon showed less pronounced, but significant comitogenic activity in concentrations of 0.25×10^{-9}-0.25×10^{-11} μg/ml. Cortagen showed no comitogenic activity.

Vilon in concentrations of 0.25×10^{-3}-0.25×10^{-4} μg/ml and 0.25×10^{-9}-0.25×10^{-12} μg/ml potentiated the comitogenic effect of rIL-1β (Fig. 2). Epithalon produced a less pronounced modulating effect in concentrations of 0.25×10^{-9}-0.25×10^{-11} μg/ml. Cortagen did not modulate (sometimes inhibited) comitogenic activity of rIL-1β.

These results are particularly interesting from the viewpoint of the regulatory peptides continuum theory

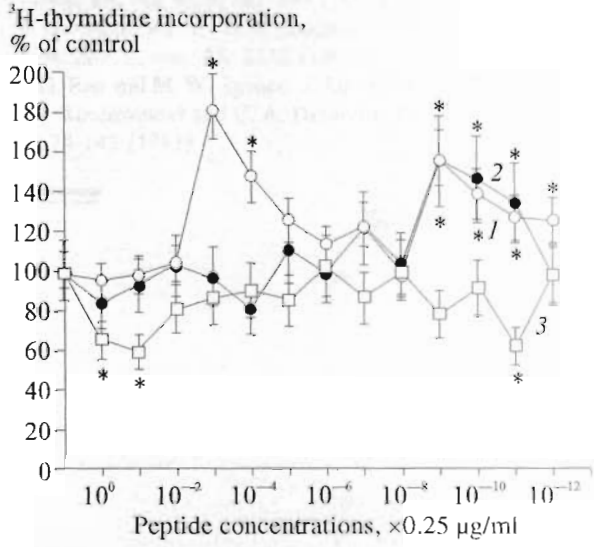

Fig. 2. Modulating effects of Vilon (*1*), Epithalon (*2*), and Cortagen (*3*) on comitogenic activity of interleukin-1β. *$p<0.05$ compared to the control (concanavalin A and interleukin-1β without peptides).

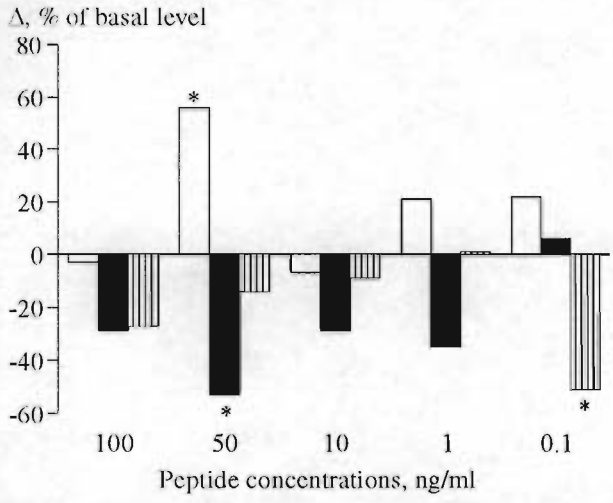

Fig. 3. Effects of Vilon (light bars), Epithalon (dark bars), and Cortagen (cross-hatched bars) on activity of neutral sphingomyelinase in mouse thymocyte membranes. *$p<0.05$ compared to basal activity.

[1], according to which mutual modulation of endogenous bioregulators ensures a wide spectrum of their biological effects on physiological functions of the organism.

The most important result in the studies of the possibility of utilization of the sphingomyelin signal transduction pathway characteristic of IL-1β is that Vilon (structural fragment of thymic peptides), but not Epithalon or Cortagen, in a concentration of 50 ng/ml, significantly stimulated activity of neutral sphingomyelinase (Fig. 3). Vilon in concentrations of 1 and 0.1 ng/ml also activated this enzyme (insignificantly). Epithalon and Cortagen did not activate neutral sphingomyelinase, while in low concentrations they even inhibited this enzyme (basal specific activity of neutral sphingomyelinase was 0.23 nmol ^{14}C-sphingomyelinase/mg protein/min). Hence, we showed that Vilon stimulated activity of neutral sphingomyelinase either by modulating transduction of known signals from bioactive substances via the sphingomyelin pathway or by directly transferring its information into thymocytes via this pathway.

These data confirm high biological activity of Vilon and Epithalon (to a lesser extent) as polyfunctional immunomodulators [9], direct thymomimetics, and modulators of IL-1β effect. These findings suggest that these short peptides are lymphocyte-activating factors comparable with classical growth factors, such as immunomodulating cytokines. The data on modulation or direct signal transduction from Vilon to target cell via signal transduction pathway typical of immunoregulatory cytokines are very important; no analogs have been heretofore described. These results open new vistas in the use of peptide preparations for correction of impaired defense mechanisms with addressed modulation of the pharmacological target (activity of neutral sphingomyelinase) in immunocompetent cells.

REFERENCES

1. I. P. Ashmarin and M. F. Obukhova, *Biokhimiya*, **51**, No. 4, 531-545 (1986).
2. E. A. Korneva, S. N. Shanin, and E. G. Rybakina, *Ros. Fiziol. Zh.*, **86**, No. 3, 292-302 (2000).
3. E. G. Rybakina, N. N. Nalivaeva, I. Yu. Pivanovich, *et al.*, *Ibid.*, 303-311.
4. K. A. Dressler, S. Mathias, and R. N. Kolesnick, *Science*, **255**, 1715-1718 (1992).
5. Y. A. Hannun, *J. Biol. Chem.*, **269**, No. 5, 3125-3128 (1994).
6. R. N. Kolesnick and D. Golde, *Cell*, **77**, 325-328 (1994).
7. S. Mathias, A. Younes, Chu-Cheng Kan, *et al.*, *Science*, **259**, No. 22, 519-522 (1993).
8. F. R. McKenzie, *Basic Techniques to Study G-Protein Function*, Stockholm (1993).
9. V. G. Morozov and V. Kh. Khavinson, *Int. J. Immunopharmacol.*, **19**, No. 9/10, 501-505 (1997).
10. N. N. Nalivaeva, E. G. Rybakina, S. N. Shanin, *et al.*, *Biochem. Soc. Trans.*, **25**, 214S (1997).
11. B. G. Rao and M. W. Spence, *J. Lipid Res.*, **17**, 506-515 (1976).
12. L. J. Rosenwasser and C. A. Dinarello, *Cell Immunol.*, **63**, No. 1, 134-142 (1981).