Evaluation of the Effect of β-Endorphin on IL-4 and γ-IFN Production by CD4+ Lymphocytes

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β-Endorphin stimulates phytohemagglutinin-induced production of IL-4 and does not modify the production of γ-IFN in nonfractionated leukocyte suspension. In a culture of purified CD4+ T-cells, β-endorphin does not modify the levels of IL-4 and γ-IFN, but stimulates the production of IL-4 and inhibits γ-IFN production after addition of monocytes to CD4+ lymphocytes. Stimulation of IL-4 synthesis by β-endorphin is mediated by the cycloxygenase cycle products. Hence, β-endorphin shifts T-helper polarization towards Th2 cells with subsequent predominance of the humoral form of the immune response.

Key Words: *β-endorphin; interleukin-4; γ-interferon; CD4+ cells; monocytes*

Three main classes of peptides involved in immunoregulation are heretofore described: peptides of the thymus, bone marrow, and neuropeptides. The majority of neuropeptides are synthesized in the nervous system cells by cleavage of large precursor molecules and are released into the blood, thus realizing the relationship between the CNS and periphery [9]. Cleavage of a large molecule of proopiomelanocortin (POMC) precursor results in the formation of a group of peptide hormones (adrenocorticotropic hormone, β-lipotropin, melanocyte-stimulating hormone, β-endorphin) characterized by a wide spectrum of biological properties. β-Endorphin is the most active and polyfunctional representative of POMK peptides. In addition to the effects exerted in the CNS (regulation of pain and some psychoemotional states), β-endorphin is characterized by pronounced immunomodulating activity, the mechanism of this effect is still little studied. Virtually all populations of immune cells serve as the targets for β-endorphin. The presence of opioid receptors of the three main classes (μ, δ, κ) and nonopioid receptor on their surface was proven by the radioligand binding method and detection of the corresponding RNA [2,6,8]. β-Endorphin modulates proliferation of lymphocytes and production of IL-2, IL-4 and γ-IFN [1,10]. However, published data were obtained mainly on the mononuclear fraction, which insufficiently shows possible effects of opioid peptides on cooperation and interaction between immune system cells.

CD4+ T-cells (helpers) act as immune response inductors. During the formation of effector clones under the effect of factors, produced by activated natural immune cells (monocytes, macrophages, dendritic cells), CD4+ cells are differentiated into two subpopulations (Th1/Th2) differing by the profile of synthesized cytokines. Th1 cells produce γ-IFN, IL-2, TNF-α, and are involved in cell-mediated response, while Th2 cells produce IL-4, IL-5, IL-10, IL-13, and mediate humoral immune response.

We studied the effect of β-endorphin on the production of IL-4 and γ-IFN by CD4+ lymphocytes in a nonfractionated leukocyte suspension in the presence of monocytes and in a fraction of purified CD4+ cells.

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MATERIALS AND METHODS

The study was carried out on peripheral venous blood leukocytes from healthy male volunteers aged 19-35 years. Nonfractionated leukocyte suspension was prepared by 2-h precipitation of heparin-treated venous blood at 37°C. The upper layer of plasma with leukocytes was then collected, centrifuged at 400*g* for 20 min, and suspended in complete nutrient medium (CNM) prepared on the basis of medium 199 and supplemented with 10 mM HEPES (Sigma), 2 mM L-glutamine (Sigma), 100 μg/ml gentamicin, and 10% FCS. The cells were cultured with phytohemagglutinin (PHA; Sigma) in the suboptimal (2.5 μg/ml) concentration in plastic 96-well round-bottom plates in humid atmosphere with 5% CO_2 at 37°C for 48 h. Each culture contained 2×10^5 cells in 0.2 ml CNM. Supernatants were collected, centrifuged, and stored frozen at -20°C. The concentrations of IL-4 and γ-IFN were measured by ELISA using Vector-Best kits according to manufacturer's instruction on a Uniplan spectrophotometer (Picon) at λ=450 nm. Analytical sensitivity of the test systems was 2 pg/ml. β-Endorphin (δ/μ-opiate receptor agonist; Sigma) was used in concentrations of 10^{-7}-10^{-10} M, sodium diclofenac in a concentration of 25 μg/ml, and anti-IL-1RACP (Chemicon Int.) in a concentration of 100 ng/ml.

$CD4^+$ T-cells were isolated using magnetic Dynabeads M-450 CD4 (Invitrogen) by adding 2×10^7 particles to the cell suspension ($1\text{-}5\times10^7$ cells/ml). After 20-min incubation at 2-8°C on a mixer, the tube with the suspension was placed into a magnetic holder for 2-3 min. Isolated cells were washed 5 times in phosphate buffer with 2% FCS. Monocytes were added to the wells as follows: nonfractionated cell suspension was placed in the plate wells, incubated for 1 h at 37°C, the supernatant with nonadherent cells was removed, and 2×10^5 $CD4^+$ T-cells in 0.2 ml CNM was added to monocytes adhered to the well walls. Each culture contained $10\text{-}15\times10^3$ monocytes, the monocyte/$CD4^+$ cell ratio was comparable to that in nonfractionated leukocyte suspension.

Statistical analysis was carried out using paired one-way analysis of dispersions and paired Student's *t* test.

RESULTS

Addition of PHA (2.5 μg/ml) to the cultures slightly modified the production of IL-4 in nonfractionated leukocyte suspension (Fig. 1). The production of γ-IFN increased significantly in the presence of PHA. β-Endorphin in concentrations of 10^{-7}-10^{-8} M stimulated PHA-induced production of IL-4 and did not modify spontaneous production of the studied cytokine. No effect of β-endorphin on spontaneous and PHA-induced production of γ-IFN in nonfractionated leukocyte suspension was detected.

β-Endorphin stimulating the production of IL-4 in leukocyte fraction did not modify its level in the culture of $CD4^+$ cells (Table 1). Addition of monocytes to $CD4^+$ lymphocytes led to stimulation of IL-4 production under the effect of β-endorphin to the level observed in nonfractionated cultures. The level of γ-IFN production by purified $CD4^+$ lymphocytes did not change in the presence of β-endorphin. However, β-endorphin significantly reduced

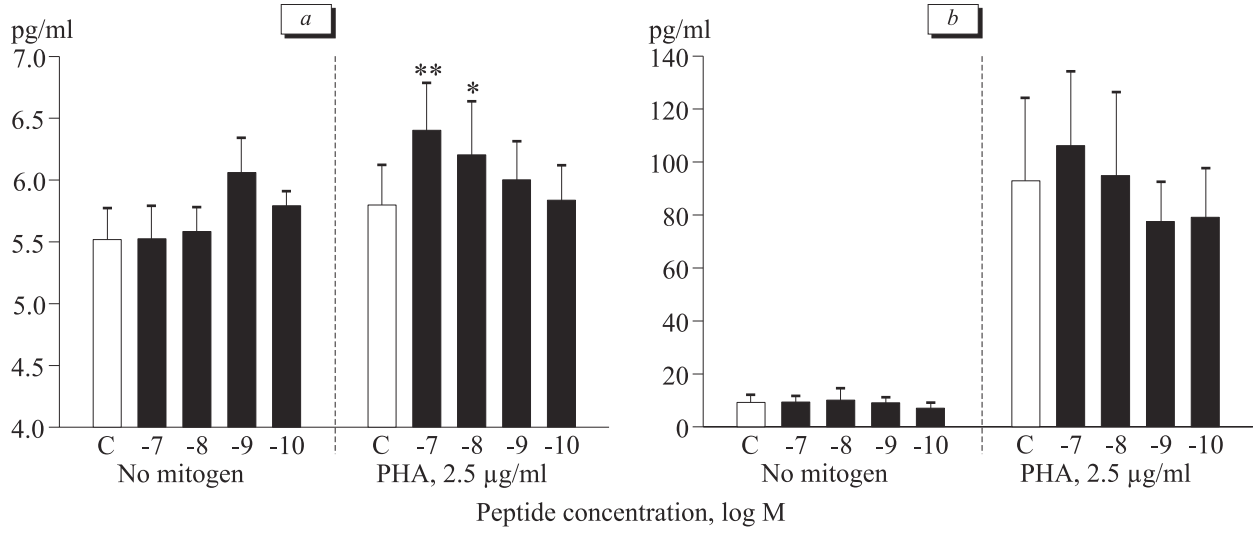

Fig. 1. Effect of β-endorphin on spontaneous and PHA-induced production of IL-4 (*a*) and γ-IFN (*b*) in nonfractionated leukocyte suspension (*n*=8). C: control. *$p<0.05$, **$p<0.01$ compared to the control.

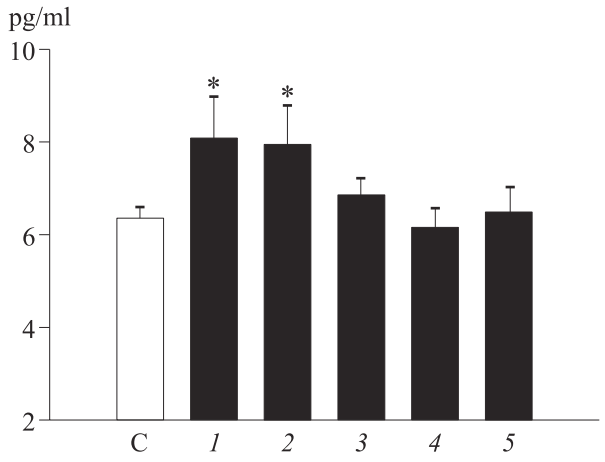

Fig. 2. Effect of β-endorphin on PHA-induced production of IL-4 in the presence of anti-IL-1RACP and blocking of prostaglandin synthesis by sodium diclofenac (n=7) in nonfractionated leukocyte suspension. *1*) β-endorphin, 10^{-7} M; *2*) β-endorphin+anti-IL-1RACP; *3*) anti-IL-1RACP; *4*) β-endorphine+sodium diclofenac; *5*) sodium diclofenac. C: control. *$p<0.05$ compared to the control.

the synthesis of γ-IFN after addition of monocytes to $CD4^+$ lymphocytes.

Hence, the effects of β-endorphin on PHA-induced production of IL-4 and γ-IFN by $CD4^+$ lymphocytes depend on the presence of the monocyte fraction in the cultures determining the direction of the effect of β-endorphin on Th1/Th2 lymphocyte population.

Of numerous factors produced by monocytes, IL-1β and arachidonic acid derivatives, *e.g.* prostaglandin E_2, most of all contribute to the regulation of IL-4 production. The production of both factors is stimulated by β-endorphin [10]. Blockade of IL-1β receptor does not change β-endorphin stimulation of PHA-induced production of IL-4 by nonfractionated leukocytes, while blockade of prostaglandin synthesis with sodium diclofenac abolished stimulation of IL-4 production (Fig. 2).

TABLE 1. Effect of β-Endorphin (10^{-7} M) on PHA-Induced (2.5 μg/ml) Production of IL-4 and γ-IFN by $CD4^+$ Lymphocytes

Cell fraction	Cytokine concentration, pg/ml	
	IL-4 (n=9)	γ-IFN (n=9)
$CD4^+$ lymphocytes		
control	5.10±1.10	66.24±16.51
β-endorphin	5.88±1.61	59.33±11.38
$CD4^+$ lymphocytes+monocytes		
control	6.02±1.06	168.72±27.60
β-endorphin	9.42±1.76*	131.36±20.30*

Note. *$p<0.05$ compared to the control.

Hence, the results indicate the key role of natural immunity cells in β-endorphin regulation of IL-4 and γ-IFN production by $CD4^+$ lymphocytes. It seems that the cycloxygenase cycle products, primarily prostaglandin E_2, play the key role in opioid-mediated regulation of IL-4 synthesis and hence, lead to polarization of helpers towards Th2 cells. It was previously shown that β-endorphin and morphine abolish the inhibitory effect of prostaglandin E_1 on IgE-mediated release of serotonin from mast cells. Addition of antibodies to IL-1 and β-endorphin into and removal of monocytes from cell cultures leads to suppression of immunoglobulin synthesis [5]. On the other hand, it was shown that β-endorphin directly stimulates the production of IL-4 by mouse $CD4^+$ lymphocytes irrespective of IL-1 and IL-6 [10]. Importantly that opioid peptides, no doubt, directly modify functional activity of lymphocytes, because these cells carry opiate receptors on their surface, the expression of these receptors increasing many-fold after mitogen stimulation of the cells (this fact explains the β-endorphin effect on IL-4 production only in PHA-stimulated cultures) [7]. However, we think that the presence of monocytes and mediators produced by them, specifically prostaglandins, is an obligatory condition for stimulation of IL-4 synthesis under the effect of β-endorphin.

The detected suppression of γ-IFN level in the $CD4^+$ cells+monocytes system indicates the suppressor effect of β-endorphin on the production of γ-IFN mediated by monocytes (similarly as for IL-4). The absence of analogous effect in the leukocyte suspension can be attributed to the presence of few $CD8^+$ and NK cells in it, which can produce γ-IFN [6], and of neutrophils which can produce γ-IFN under certain conditions [5]. Published data on the opioid effects on γ-IFN production are contradictory and disputable.

Various authors observed stimulatory and inhibitory effects mainly on mixed cell fraction [4]. For example, the production of γ-IFN was suppressed by a mechanism realized through μ-receptor on monocytes and stimulated through a nonopioid receptor. We think that the production of γ-IFN by the main producers ($CD4^+$ lymphocytes) is inhibited in the presence of β-endorphin, which inhibits the development of cellular response and directs the immune response by the Th2 pathway.

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