

CLINICAL SIGNIFICANCE OF MEASUREMENT OF INTERLEUKIN 4 AND INTERLEUKIN 5 SERUM CONCENTRATIONS IN BRONCHIAL ASTHMA

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Summary: Cytokines IL-4 and IL-5 participate in the chronic inflammation process in the atopic airway diseases. Our study is aimed at determining correlation between IL-4 and IL-5 serum concentrations and severity of the disease in patients with bronchial asthma. The total of 65 patients with asthma is classified into three groups: 1. patients with mild, 2. moderate and 3. severe asthma. The control group comprised 10 healthy individuals. Measurement of IL-4 and IL-5 serum concentrations was performed according to ELISA method. Minimal detectable concentrations of IL-4 and IL-5 were 0.27 pg/mL and 4 pg/mL, respectively. IL-4 ($Z = 2.245$, $p = 0.025$) and IL-5 ($Z = 2.139$, $p = 0.032$) serum concentrations are significantly lower in healthy individuals in comparison to the individuals with asthma. Comparison of the study results between the groups (mild, moderate and severe asthma) evidenced that IL-4 serum concentrations in the group with moderate ($Z = 2.604$, $p = 0.009$) and severe asthma ($Z = 2.658$, $p = 0.008$) were statistically significantly higher in comparison to the group with mild asthma. IL-5 serum concentrations in the group with moderate ($Z = 3.910$, $p = 0.000$) and severe asthma ($Z = 4.037$, $p = 0.000$) were statistically significantly higher in comparison to the group with mild asthma. Further studies on the role of IL-4 and IL-5 in the process of chronic inflammation in asthma are needed.

Key words: asthma, interleukin 4, interleukin 5, asthma severity

Introduction

It has been widely accepted that chronic inflammation of the airway mucosa plays major role in pathogenesis of the bronchial asthma, in which, numerous cells have important roles, including mastocytes, eosinophils and T-lymphocytes. Inflammatory cells produce numerous mediators and cytokines which provide for the local mechanism that induces, enhances and modulated the ongoing modulation. Interleukin 4 (IL-4) and interleukin 5 (IL-5) have important roles in these events.

Interleukin 4 is immunoregulatory cytokine produced by Th2 lymphocytes, mast cells, basophiles and subpopulation of NK cells. It is a multifactorial cytokine acting as a mitogen for both B and T-lymphocytes and induces transcription of the germinative line

of IgC γ 1 and C ϵ genes, which represents introduction into the specific immunoglobulin rearrangement and induces positive regulation of expression of MHC II molecules, CD23 and Th1 on B cells as well as IL-4 receptors on B and T cells. *In vitro*, IL-4 is necessary for differentiation of the naive CD-positive T-cells within the Th2 subpopulation secreting IL-4, IL-5, IL-6, IL-10 and IL-13 (1). Although IL-4 induces IgE synthesis and enables the immediate type of hypersensitivity reaction, there is certain evidence suggesting *in vitro* and *in vivo* anti-inflammatory effects of IL-4 (2). Interleukin 4 leads to increased expression of VCAM1 on the endothelial cells whose ligand VLA-4 is specifically expressed on the eosinophils and lymphocytes which enables characteristic VCAM-1/VLA-4 transmigration of eosinophils and eosinophilic tissue infiltration, characteristic for chronic inflammation in individuals with bronchial asthma. Interleukin 4 mostly expresses antagonizing effects with interferon gamma to expression of numerous cytokine genes in the monocytes, thus leading to inhibition and suppression of genes for TNF-alpha, IL-1 beta, IL-6 and IL-CD23, 15 lipooxygenase, IL-1 receptor antagonist, as well as both types (type I and type II) of IL-1 receptors (3).

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Interleukin 5 is 20 kD cytokine that belongs to 4-alpha helix cytokine family. Together with IL-3, IL-4 and GM-CSF it is found in the human chromosome 5. T-cells, mast cells and eosinophils are the sources of IL-5. Interleukin 5 is secreted only by the activated, not by inactive T-cells, specifically Th2 subpopulation. Using this mediator, lymphocytes regulate function of eosinophils. On the other hand, autocrine production of IL-5 by eosinophils contributes to the chronicity of inflammation through constant mobilization and activation of these cells (4). It is believed that circulating cells tend to gain increased capacity to secrete IL-5 when they come into the lungs, where they are stimulated by other cytokines as well, such as IL-2 and IL-17 (5). This mediator performs its functions through the specific IL-5 receptor expressed on the limited number of the target cells (4). Due to the significance of IL-5 in chronic inflammation in asthma and its effects on the eosinophils as the basic cells of chronic inflammation in the airways, IL-5 functions may be summarized as follows: maturation, migration and rapid release of eosinophils from the bone marrow and terminal differentiation of precursor cells; transmigration of eosinophils across vascular endothelium and prolongs survival of eosinophils reducing apoptosis.

The study is aimed at determining possible differences in IL-4 and IL-5 serum concentrations in different degrees of severity of the bronchial asthma.

Material and Methods

The study included the total of 65 patients with bronchial asthma and control group composed of 10 healthy individuals. Based on the severity of the clinical picture, the patients were classified into three groups:

1. Mild bronchial asthma (intermittent and mild, persistent) (n=19).
2. Moderate asthma (moderately severe, persistent) (n=11).
3. Severe persistent asthma (n=35).

Diagnosis of bronchial asthma was established based on the adopted criteria of the International consensus on diagnosis and therapy of bronchial asthma from 1995 (revised in 2002) (6).

Asthma classification according to severity of the disease is determined based on the clinical findings and pulmonary function findings as measured using the apparatus Autospir Discom-14 Chest Corporation Tokyo, Japan.

IL-4 and IL-5 serum concentration measurement is performed according to ELISA method (commercial kit Cytoscreen Immunoassay KIT Human IL-4 and IL-5 Biosource International, USA), pursuant to the manufacturer's instructions. Minimal detectable concentrations of IL-4 and IL-5 are 0.27 pg/mL, and 54 pg/mL, respectively, also based on the manufacturer's recom-

mendations (7). Measurement of IL-4 and IL-5 serum concentrations was performed according to ELISA method.

Statistics

Database and statistical analysis were performed using SPSS 7.5 package for Windows. Analysis of the obtained data, i.e., comparison of differences between the two groups with respect to the non-parametric properties was performed using chi square test and Mann-Whitney test. In case of comparison of differences between three or more groups of data, variance analysis according to Kruskal Wallis was used (8, 9).

Results

The study included the total of 65 patients with asthma with approximately the same distribution of both males (32 subjects, 49.23%) and females (33 subjects, 50.77%) and average age of mean = 34.73 years. The control group comprised 10 healthy individuals. Serum concentrations of IL-4 ($Z = 2.245$, $p = 0.023$) and IL-5 ($Z = 2.139$, $p = 0.032$) were significantly lower in healthy individuals in comparison to the group with bronchial asthma (see *Table I*).

Comparison of the study results between the groups (mild, moderate, severe asthma) evidenced that serum concentrations of IL-4 and IL-5 in groups with moderate and severe asthma were statistically significantly higher in comparison to the group with mild asthma. Significantly higher serum concentrations of IL-4 were found in the group of patients with moderate asthma in comparison to the group with mild asthma ($Z = 2.604$, $p = 0.009$). Concentrations of IL-4 were statistically significantly higher in the group with severe asthma in comparison to the group with mild asthma

Table I. IL-4 and IL-5 concentrations in patients with bronchial asthma and healthy individuals.

	CONTROL GROUP (n=10)	ASTHMA		
		Mild (n=19)	Moderate (n=11)	Severe (n=35)
IL-4				
Mean	0.15	0.45	1.38	2.47
SD	0.22	1.13	3.23	11.94
Med	0.00	0.00	0.51	0.39
Min	0.00	0.00	0.00	0.00
Max	0.53	4.41	11.10	71.00
IL-5				
Mean	10.70	8.62	60.00	40.70
SD	11.82	15.74	46.98	39.70
Med	7.23	1.88	37.40	30.16
Min	0.00	0.00	10.50	0.00
Max	31.54	64.30	126.71	156.02

($Z = 2.658$, $p = 0.008$). No statistically significant difference in IL-4 serum concentrations was found in patients with severe asthma in comparison to the group with moderate asthma ($Z = 0.684$, $p = 0.494$) (Table 1).

Significantly higher IL-5 serum concentrations were found in the group of patients with moderate asthma in comparison to the group with mild asthma ($Z = 3.910$, $p = 0.000$). As for the group with severe asthma, IL-5 serum concentrations were statistically significantly higher in comparison to the group with mild asthma ($Z = 4.037$, $p = 0.000$). Finally, no statistically significant difference in IL-5 serum concentrations was found between patients with severe and moderate asthma ($Z = 1.236$, $p = 0.216$) (Table 1).

Discussion

Our study is aimed at determining correlation between IL-4 and IL-5 serum concentrations and severity of the disease, i.e., whether measurement of the cytokines in the serum may have diagnostic value in assessment of the degree of disease severity as well as predictive value.

There is only small number of papers on measurement of serum IL-4 and IL-5. Motojima et al (7) determined IL-5 serum concentrations in 78 asthma patients (the control group comprised 30 healthy individuals). Results of the study revealed that IL-5 serum concentration in asthma patients was statistically significantly higher in comparison to IL-5 concentration values in the healthy controls. Comparison of the study results between the groups (mild, moderate and severe asthma) shows that IL-5 serum concentrations are significantly higher in the group with moderate and severe asthma in comparison to the group with mild asthma. IL-5 serum concentrations obtained during the remission period were significantly lower than IL-5 values measured at the time of disease exacerbations.

Alexander et al (10) measured IL-5 serum concentrations in 29 patients with stable steroid-dependent severe asthma and evidenced detectable IL-5 serum concentration in 15 patients (53%), while it was completely undetectable in the healthy controls.

Oymar et al (11) determined IL-5 serum concentrations in 44 children aged between 12 and 84 months with mild and moderate asthma both during symptomatic and asymptomatic phase of the disease. Detectable IL-5 values were found in 8 out of 15 children with acute asthma, while all the samples obtained from children with stable asthma as well as from the controls were negative.

Matsumoto et al (12) determined serum IL-4 concentrations as well as concentrations of soluble

FcεRII and soluble IL-2 receptor in 77 individuals with mild and moderate asthma and in 75 healthy controls. They evidenced higher values of all the above-mentioned immunological markers in sera of the asthma patients in comparison to the healthy controls. Hashimoto et al. (13) also found significantly higher IL-4 serum concentrations in patients with bronchial asthma in comparison to those obtained in healthy controls.

The study conducted by Hacken et al (14) also verified significantly higher IL-4 and IL-5 serum concentrations in 17 patients with asthma in comparison to concentrations of the cytokines in healthy controls, however they have failed to evidence correlation between IL-4 and IL-5 concentrations and parameters of clinical manifestations of asthma.

In our study, significantly lower IL-4 and IL-5 serum concentrations were found in the group of healthy controls in comparison to the group with bronchial asthma. IL-4 and IL-5 serum concentrations in patients with moderate and severe asthma were significantly higher in comparison to those obtained in the group with mild asthma.

Lack of significant difference in IL-4 and IL-5 serum concentrations between patients with moderate and severe asthma supports the attitudes of the majority of authors considering that the groups with these forms of asthma may be practically treated during the studies as one common group of patients with prominent and advanced inflammation which is significantly different from inflammation found in mild asthma.

These results might be indicative of different degrees of the disease (disease severity) with their underlying degrees of chronic inflammation also being different, and IL-4 and IL-5 as possible markers. The above concept is consistent with already accepted fact that order of events in asthma-initial inflammation (regardless of possible allergic background) characterized by cells and mediators (cytokines or others), followed by hyperreactivity of airways and disease manifestations (clinical picture).

It may be concluded that the results of our study are consistent with other results reported so far, suggesting that cytokines IL-4 and IL-5 obviously represent good markers of chronic inflammation of the lower airways in bronchial asthma. Their application for the diagnostic as well as predictive purpose may be reasonably recommended, however only within the research projects for the time being. Additional studies on the role of IL-4 and IL-5 in chronic inflammation associated with asthma are needed.

Acknowledgement: The study was sponsored by the Hemofarm Pharmaceutical Company.

KLINIČKI ZNAČAJ MERENJA KONCENTRACIJE INTERLEUKINA 4 I INTERLEUKINA 5 U SERUMU U BRONHIJALNOJ ASTMI

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Kratak sadržaj: Citokini IL-4 i IL-5 učestvuju u procesu hronične inflamacije u atopijskim bolestima disajnih puteva. Cilj ove studije je bio da se utvrdi povezanost koncentracije IL-4 i IL-5 u serumu sa težinom bolesti kod bolesnika sa bronhijalnom astmom. Ukupno 65 bolesnika sa astmom je svrstano u tri grupe i to sa blagom, umerenom i teškom astmom. U kontrolnoj grupi je bilo deset zdravih osoba. Koncentracije IL-4 i IL-5 u serumu su merene pomoću ELISA metode. Minimalna detektabilna koncentracija za IL-4 je bila 0,27 pg/mL, a za IL-5 je 4 pg/mL. Zdrave osobe su imale značajno nižu koncentraciju IL-4 ($Z = 2,245$, $p = 0,025$) i IL-5 ($Z = 2,139$, $p = 0,032$) u serumu u odnosu na osobe sa astmom. Poređenje rezultata ispitivanja po grupama (blaga, umerena i teška astma) pokazuje da su koncentracije IL-4 u serumu u grupi sa umerenom ($Z = 2,604$, $p = 0,009$) i teškom astmom ($Z = 2,658$, $p = 0,008$) statistički značajno više u odnosu na grupu sa blagom astmom. Koncentracije IL-5 u serumu u grupi sa umerenom ($Z = 3,910$, $p = 0,000$) i teškom astmom ($Z = 4,037$, $p = 0,000$) statistički su značajno više u odnosu na grupu sa blagom astmom.

Cljučne reči: astma, interleukin 4, interleukin 5, težina astme

References

- Huang H, Hu-Lu J, Chen H, Ben-Sasson SZ, Paul WE. IL-4 and IL-13 Production in Differentiated T Helper Type 2 Cells Is Not IL-4 Dependent. *The J Immunol* 1997; 159: 3731-38.
- Ohmori Y, Hamilton AT. IL-4 Induced STAT6 Suppresses IFN- γ -Stimulated STAT1-Dependent Transcription in Mouse Macrophages. *J Immunol* 1997; 159: 5474-82.
- Dickensheets HL, Donnelly PR. IFN- γ and IL-10 Inhibit Induction of IL-1 Receptor Type I and Type II Gene Expression by IL-4 and IL-13 in Human Monocytes. *J Immunol* 1997; 159: 6226-33.
- Ergan RW, Umland SP, Cuss FM, Chapman RW. Biology of interleukin-5 and its relevance to allergic disease. *Allergy* 1996; 51:71-81.
- Lai C KW, Ho ASS, Chan CHS, Tang J, Leung JCK, Lai KN. Interleukin-5 messenger RNA expression in peripheral blood CD4+ cells in asthma. *J Allergy Clin Immunol* 1996 (97): 1320-6.
- National Heart Lung and Blood Institute National Institutes of Health. Global Strategy for Asthma Management and Prevention. NHLBI/WHO Workshop Report NIH Pub No 95-3659, 1995, Revised 2002, 2.
- Motojima S, Akutsu I, Fukuda T, Makino S, Takatsu K. Clinical significance of measuring levels of sputum and serum ECP and serum IL-5 in bronchial asthma. *Allergy* 1993; 48: 98-106.
- Jekel JF, Elmore JG, Katy DL. *Epidemiology Biostatistics and Preventive Medicine*. Philadelphia, WB Saunders Company, 1996.
- Erić-Marinković J, Dotlić R, Janošević S, Kocev N, Gajić M, Ille T, Stanisavljević D, Babić D. Statistika za istraživače u oblasti medicinskih nauka. Beograd, Medicinski fakultet, 2001.
- Alexander AG, Barkans J, Moqbel R, Barnes NC, Kay B, Corrigan CJ. Serum interleukin 5 concentrations in atopic and non-atopic patients with glucocorticoid-dependant chronic severe asthma. *Thorax* 1994; 49: 1231-3.
- Oymar K, Elsayed S, Bjerknes R. Serum eosinophil cationic protein and interleukin-5 in children with bronchial asthma and acute bronchiolitis. *Pediatr Allergy Immunol* 1996; 7 (4): 180-6.
- Matsumoto K, Taki F, Miura M, Matsuzaki M, Takagi K. Serum levels of soluble IL-2R, IL-4 and soluble Fc epsilon RII in adult bronchial asthma. *Chest* 1994; 105 (3): 681-6.
- Hashimoto S, Amemiya E, Tomita Y, Arai K, Yamaguchi M, Horie T. Elevation of soluble IL-2 receptor and IL-4 and nonelevation of IFN-gamma in sera from patients with allergic asthma. *Ann Allergy* 1993; 71 (5): 455-8.
- Hacken NH, Oosterhoff Y, Kauffman HF, Guevarra I, Satoh T, Tollerud DJ, et al. Elevated serum interferon-gamma in atopic asthma correlates with increased airways responsiveness and peak expiratory flow variation. *Eur Resp J* 1998; 11 (2): 312-6.

Received: October 15, 2003

Accepted: December 18, 2003