MICROBIOLOGY AND IMMUNOLOGY

IL-5 Expression in the Sputum of Patients with Bronchial Asthma

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Expression of IL-5 mRNA and the content of IL-5 in the sputum of patients with asthma of different severity were studied before and after treatment. The expression of IL-5 mRNA in mild asthma differed from that in severe and moderate asthma before and after treatment. The level of IL-5 before therapy was different in patients with mild and severe disease. In patients with severe asthma the level of IL-5 differed before and after treatment.

Key Words: *interleukin-5; asthma*

Asthma is a chronic inflammatory disease of the airways involving many cells: mast cells, eosinophils, T cells, *etc.* Atopic asthma is associated with chronic eosinophilic inflammation, whose mechanism is still not quite clear [5]. Eosinophils damage the bronchial epithelium promoting the development and persistence of inflammation. These cells are present in great numbers in the bronchoalveolar lavage fluid, biopsy specimens, and autopsy material from patients with exacerbation and even remission [4].

A critical role in the development of inflammation is played by IL-5 and other factors (IL-3, GM-KSF) determining accumulation and activation of eosinophils [12], which results in the release of proinflammatory agents in the infiltration zone [7]. Association of IL-5 C-703T gene polymorphism with atopic asthma was reported [3]. Since IL-5 selectively activates eosinophils in humans and is the main cytokine associated with eosinophilic inflammation [2], it can play a key role in the development of eosinophilic inflammation in asthma.

Like IL-4, IL-5 cytokine is produced by CD4 T cells [6]. Enhanced expression of IL-5 mRNA was found in bronchial biopsy specimens from asthmatics. This parameter directly correlates with the severity of the disease, decrease in the forced expiratory volume over the first second (VFE_1), and peak expiratory flow rate (PEFR) [10]. The data on the content of this cytokine in the sputum of asthmatic patients are contradictory: according to some reports IL-5 level depends on the severity of asthma [10], while according to others it does not [1]. We measured IL-5 mRNA expression by sputum cells and elucidated whether this parameter correlated with the results of direct measurements of IL-5 level in the sputum by enzyme immunoassay.

We studied the expression of IL-5 mRNA and IL-5 level in the sputum in patients with mild, moderate, and severe asthma.

MATERIALS AND METHODS

Sixty-six adult patients (mean age 25 years) with bronchial asthma were examined, 7 of these had mild atopic asthma, 40 had moderate persistent, and 19 severe persistent form (according to clinical and functional studies). The diagnosis and evaluation of the disease severity were carried out using GINA 2002 tests. Pa-

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tients were treated with fluticasone propionate in doses depending on the disease severity: 250 μg/day in mild asthma, 500 μg/day in moderate, and 1000 μg/day in severe asthma. The duration of treatment was 12 weeks in all groups. The main criterion of treatment efficiency was PEFR. The patients were examined before and after therapy. IL-5 level and the expression of IL-5 mRNA in the sputum were compared in different groups and before and after therapy in the same group.

Induced sputum was collected before and after treatment with inhalation corticosteroids. Sputum specimens (up to 5 ml) were collected in plastic containers. The sputum was treated with mucolysin (dithiorithiol solution, Central Institute of Epidemiology, Ministry of Health of Russian Federation, Moscow).

The cell suspension was transferred onto slides, fixed with ethanol, stained with Azur-eosin, and the relative count of eosinophils in induced sputum was determined.

Total RNA was isolated using Ribo-zol kits (Central Institute of Epidemiology, Ministry of Health of Russian Federation) in accordance with manufacturer's instruction. cDNA was obtained by reverse transcription of total RNA using oligo-dT12-18 primers and M-MuLV reverse transcriptase.

The following primers were used for RT-PCR: 5'-GCT TCT GCA TTT GAG TTT GCT AGC T: 3'-TGG CCG TCA ATG TAT TTC TTT ATT (7.5 μM each, R&D Systems, Inc). PCR analysis was carried out by the standard method with AmpliSense-200-n kit (Central Institute of Epidemiology, Ministry of Health of Russian Federation). The amplicon size was 271 b. p. Synthetic double-stranded DNA (R&D Systems, Inc) was used as positive control, the product size was 340 b. p. DNA amplification was carried out on a Tertsik device (DNK-Tekhnologiya) according to the following protocol: denaturation 45 sec at 94°C, annealing 45 sec at 55°C, and synthesis 45 sec at 72°C (a total of 35 cycles).

Semiquantitative evaluation of IL-5 mRNA expression was carried out by comparing with expression of glyceraldehyde-3-phosphate dehydrogenase gene using the following primers: 5'-GGG AAG CTC ACT GGC ATG GCC TTC C; 3'-CAT GTG GGC CAT GAG GTC CAC CAC.

Amplification products were fractionated by electrophoresis in 2% agarose gel and analyzed using a videosystem and Biotest D software. The expression of IL-5 mRNA was expressed in arbitrary units.

Enzyme immunoassay was carried out using Cytelisa IL-5 kits (Cytimmune) on a standard plate spectrophotometer.

The results were statistically processed using nonparametrical Mann—Whitney test and Statistica 5.0 software.

RESULTS

The therapy with inhalation corticosteroids was effective and led to positive changes in all 3 groups (Table 1).

Expression of IL-5 mRNA in the sputum of patients with mild asthma differed from that in patients with severe and moderate asthma both before and after treatment. The groups with severe and moderate asthma did not differ by this parameter. In none groups the differences in this parameter before and after treatment were detected (Table 2).

The comparison of IL-5 level before therapy in groups with mild and severe asthma showed significant differences (Table 3). Interestingly, no significant differences in this parameter were detected after the treatment. In patients with severe asthma (but not in other groups) IL-5 levels before and after treatment were different (Table 3).

Thus, the treatment was very effective, which was indirectly confirmed by a decrease in the relative count of eosinophils in the sputum (5.6±0.7 *vs.* 15.3±1.4% in mild asthma, $p<0.05$; 10.3±2.5 *vs.* 18.6±3.4% in moderate asthma, $p<0.05$; and 14.9±1.8 *vs.* 18.3±2.5% in severe asthma, $p<0.05$). Significant changes in IL-5 level after therapy were detected only in patients with severe asthma, while the level of IL-5 mRNA was associated with the disease severity and did not change during treatment. This paradox can be explained by recently discovered constitutive expression of IL-5 mRNA and protein by epithelial cells [11]. Presumably, intensive expression of IL-5 mRNA and production of this cytokine in asthmatics, especially in patients with severe asthma, are maintained by bronchial epithelial cells (and probably are genetically determined).

Despite the absence of a clear-cut relationship between IL-5 level and disease severity, analysis of correlations demonstrated a relationship between IL-5 level and expression of IL-5 mRNA in the groups with moderate ($r=0.74$, $p=0.0012$) and severe asthma ($r=0.61$, $p=0.025$).

Presumably, the expression of IL-5 mRNA reflects T cell-mediated reaction leading to activation of eosinophils. It is known that IL-5 mRNA expression in the bronchial mucosa of asthmatics correlates with eosinophil count [5,8], while eosinophilia is directly related to the severity of the disease [5]. These data confirm regulation of atopic asthma severity by IL-5 cytokine through the mechanism stimulating eosinophilia. Treatment decreased eosinophilia, but not reduced IL-5 mRNA expression, which can be associated with mRNA production by epithelial cells.

The correlation between IL-5 level and degree of IL-5 mRNA expression during exacerbation of the

TABLE 1. Changes in Peak Expiratory Flow Rate during Therapy (*M*±*m*, % of the Best Personal Value)

Parameter	Severity of asthma		
	mild (*n*=7)	moderate (*n*=40)	severe (*n*=19)
Before therapy	89.3±3.4	69.7±5.3	53.1±2.3
After therapy	95.6±3.6*	86.5±7.7*	84.6±9.3*

Note. Here and in Tables 2 and 3: *n*: number of patients. *$p<0.05$ compared to this parameter before therapy.

TABLE 2. Expression of IL-5 mRNA in the Sputum of Patients with Asthma of Different Severity before and after Therapy (arb. units)

Parameter	Severity of asthma		
	mild (*n*=7)	moderate (*n*=40)	severe (*n*=19)
Before therapy	0.57±0.20	1.98±0.14*	2.21±0.25***
After therapy	0.71±0.18	1.93±0.13**	1.68±0.17****

Note. *$p=0.0005$, **$p=0.0009$, ***$p=0.0025$, ****$p=0.005$ compared to mild asthma.

TABLE 3. Level of IL-5 in Sputum of Patients with Asthma of Different Severity before and after Therapy (pg/ml)

Parameter	Severity of asthma		
	mild (*n*=7)	moderate (*n*=40)	severe (*n*=19)
Before therapy	17.2±6.4*	54.1±21.6	98.41±17.3^{+}
After therapy	19.8±9.7	34.2±12.5	41.6±11.9

Note. *$p=0.052$ compared to severe asthma, $^{+}p=0.019$ compared to this parameter after treatment.

disease (severe and moderate asthma) and the absence of this correlation in mild asthma indicate that some additional mechanisms can regulate the level of free cytokine in biological liquids at the level of translation or at the level of the ligand-receptor interactions in mild asthma. Soluble form of IL-5 receptor α-chain forming as a result of alternative slicing can act as such a regulator [10]. Soluble form of the receptor can regulate IL-5 gene expression and decrease the cytokine content in biological fluids due to its binding and neutralization.

REFERENCES

1. O. S. Kobyakova, M. B. Freidin, F. I. Petrovskii, *et al.*, *Byull. Eksp. Biol. Med.*, Suppl. I, 66-68 (2001).
2. L. M. Ogorodova, O. S. Kobyakova, M. B. Freidin, *et al.*, *Allergologiya*, No. 4, 32-36 (1999).
3. V. P. Puzyrev, M. B. Freidin, L. M. Ogorodova, *et al.*, *Byull. Eksp. Biol. Med.*, Suppl. I, 50-52 (2000).
4. A. G. Chuchalin, *Chronic Obstructive Diseases of the Lungs* [in Russian], Moscow, St. Petersburg (1998).
5. C. J. Corrigan and A. B. Kay, *Immunol. Today*, **13**, 501-507 (1992).
6. V. Gemou-Engesaeth, M. K. Fagerhol, M. Toda, *et al.*, *Pediatrics*, **109**, No. 2, 24 (2002).
7. R. H. Gundel, L. G. Letts, and G. J. Gleich, *J. Clin. Invest.*, **87**, 1470-1473 (1991).
8. Q. Hamid, M. Azzawi, S. Ying, *et al.*, *J. Clin. Invest.*, **87**, 1541-1546 (1991).
9. M. Humbert, C. J. Corrigan, P. Kimmitt, *et al.*, *J. Respir. Crit. Care Med.*, **156**, No. 3, 704-708 (1997).
10. D. P. Huston, *International Journal on Immunorehabilitation*, No. 7, 7-12 (1997).
11. S. Salvi, A. Semper, A. Blomberg, *et al.*, *Am. J. Respir. Cell. Mol. Biol.*, **20**, 948-991 (1999).
12. A. J. Wardlaw, R. Moqbel, and A. B. Kay, *Adv. Immunol.*, **60**, 151-266 (1995).