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Role of interleukin-17 in the disease course of different phenotypes of chronic obstructive pulmonary disease

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Abstract
Work purpose - to study of immunologic features of the disease course of different COPD phenotypes – with chronic bronchitis and emphysema, and role of interleukin-17 in the pathogenesis of COPD. 87 male patients were involved in the examination, informed consents of all of them were obtained. Average age of patients was 53,9±4,5 years. Control group consisted of 36 persons, randomized by age and sex without any signs of COPD. Patients with chronic obstructive pulmonary disease with chronic bronchitis phenotype were observed to have signs of metabolic syndrome, lipid metabolism disorders and insulin resistance, unlike patients with emphysema phenotype. Patients of both clinical phenotypes have an equal severity of airway obstruction, exacerbation frequency and systemic arterial hypertension as a concomitant disease. Patients with COPD with phenotype of chronic bronchitis and frequent exacerbations have Th17 response predomination with significant elevation of IL-17, TGF-β, IL-6 concentrations in serum and high concentration of soluble adhesion molecules along with high expression of receptors to adhesion molecules on activated lymphocytes of peripheral blood. Patients with COPD emphysema phenotype with frequent exacerbations in immune system have Th1 response predomination with significantly higher concentrations of IFN-γ and lower levels of IL-4 and IL-10, along with high level of activated lymphocytes with $CD25^+$ phenotype and low with $CD30^+$ phenotype. In both groups of patients with chronic obstructive pulmonary disease a high serum level of proinflammatory cytokines (TNF-α, IL-1β and IL-8) was observed.

Keywords: Chronic Obstructive Pulmonary Disease, clinical phenotypes, cytokines, immune response, intrleukin-17

1. Introduction
Chronic obstructive pulmonary disease (COPD), according to the WHO expert evaluation, is one of the most common human diseases. Clinical observations of the last years confirm a great variety in the disease course of COPD and its heterogeneity in clinical, functional, radiological, and pathomorphological findings that reveal different pathogenetic mechanisms of obstructive syndrome [2,4,5]. Inflammation has a key role in the pathogenesis of COPD. It has been confirmed by numerous studies that COPD patients develop significant elevation of systemic inflammatory markers – interleukin-6 (IL-6), C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), fibrinogen and total leukocyte count in the peripheral blood. Studies also show that persistent systemic inflammation predicts poorer prognosis for COPD patients. Based on this fact particular COPD phenotype has been distinguished – systemic inflammatory phenotype.
According to latest recommendations of GOLD experts [6] all the COPD patients are divided into 4 categories: A, B, C and D, depending on severity of bronchial obstruction and clinical manifestation (according to the mMRC Dyspnea Scale), COPD Assessment Test (CAT) and risk of exacerbation.
During the last 5-6 years the growing attention has been paid to the Spanish COPD Guidelines developed by Spanish Society of Pulmonology and Thoracic Surgery (M. Miravitles, 2014) [5]. which identify five clinical phenotypes, each requires different approaches to treatment: with emphysema and frequent exacerbations, with chronic bronchitis and frequent exacerbations, with mixed COPD-asthma, with emphysema and infrequent exacerbations and with chronic bronchitis and infrequent exacerbations [5]. There are also additional phenotypes: COPD and bronchiectasis, FEV1 phenotype with rapid decline, cachexia phenotype and α1- antitrypsin deficiency [2]. At the present time the most common COPD phenotypes are with rare exacerbations, COPD with frequent exacerbations and chronic bronchitis or emphysema dominance, mixed COPD-asthma and COPD with systemic inflammatory [2].

Purpose of Research

Is-to study of immunologic features of the disease course of different COPD phenotypes – with chronic bronchitis and emphysema, and role of interleukin-17 in the pathogenesis of COPD.

Materials and Methods of Research

87 male patients were involved in the examination, informed consents of all of them were obtained. Average age of patients was 53, 9±4,5 years. Exclusion criteria were acute infectious diseases, exacerbated or decompensated chronic somatic disorders. Control group consisted of 36 persons, randomized by age and sex without any signs of COPD.

Diagnosis of COPD was established according to the Order No. 555 of the Ministry of Health of Ukraine based on the anamnesis, physical examination, spirometry and post bronchodilator test, results of mMRC and CAT, roentgenography [3]. Spirometry was performed using the apparatus "Spirosift SP-5000" (Fukuda Denshi, Japan).

Levels of pro- and anti-inflammatory cytokines, IL-17A, interferon-γ (IFN-γ), content of soluble adhesion molecules sICAM-1, sVCAM were measured by sets certificated in Ukraine for ELISA testing under the methods of "Pro Con" (Russia), "Diaclone" (France), transforming growth factor-β (TGF-β) and IL-21 by "Genzyme diagnostics". Lymphocyte subpopulation with phenotypes CD30+, CD25+, CD54+, CD11b+, CD62L+ were identified using indirect immunofluorescence technique with monoclonal antibodies. For the preparation of supernatants with IL-17 cells of peripheral blood (heparinized blood diluted 1:4 was used) were incubated in the presence of a mitogen (LPS-induced synthesis) and in the cultivation medium (RPMI-1640 without a mitogen – spontaneous synthesis) for 24-48 hours in the atmosphere with 5% CO_2, t=37°C. After cultivation the samples were centrifuged for 10 minutes at 400g and the supernatant was collected for further testing.

Statistic processing of data was performed using a software package Microsoft XP Excel" and standard version of Statistical Package for the Social Sciences (SPSS) 17.0. Statistically significant was p-value of $p<0.05$.

Results and Discussion

For the identification of clinical, functional and immunologic features of different COPD phenotypes all the patients were divided into two groups: group I (48 patients) with predomination of chronic bronchitis and group II (39 patients) with predomination of pulmonary emphysema. All the patients had been confirmed to have the main diagnostic criterion for COPD – FEV1/FVC<70%, irreversible obstruction with negative bronchodilator response. Spirometry showed that forced expiratory volume during the first second (FEV1) for the I group was 62,6±1,74%, for the II group – 64,1±1,67%, interviewing showed short breath of 2 scores in both groups using mMRC scale and short breath of 10 scales in both groups using CAT. Herewith all patients were classed to the group B in accordance with the Order No. 555. Frequency of exacerbations in both groups of patients is 0,5 – 1 times per year.

It should be noted that the patients in the group I had signs of metabolic syndrome, manifested in high waist circumference, dyslipidemia with elevated triglycerides (TG) and low high-density lipoprotein (HDL) levels, systemic arterial hypertension, disorders of carbohydrate metabolism, insulin resistance and significantly elevated HOMA-IR index. In the group II systemic arterial hypertension and disorders of lipid metabolism (elevated cholesterol and low-density lipoprotein) were observed, but there were no patients with obesity (Table 1). Since hypertension was observed in both groups of patients, the level of blood pressure was also evaluated. This observation revealed that there were no significant differences in systolic and diastolic blood pressure levels, heart rate, LV mass index and disease duration between two groups of patients. Still all the patients were confirmed to have hypertension stage II.

Table 1: Basic clinical and biochemical parameters in COPD patients with different phenotypes (M±m)

Parameter	Group I (n=48)	Group II (n=39)	Probability value (p)
Duration of hypertension, years	10,2±1,2	9,5±1,3	p>0,1
Systolic BP, mmHg	165,7±2,1	162,5±2,7	p>0,1
Diastolic BP, mm Hg	98,7±2,3	95,1±2,1	p>0,1
Heart rate, b.p.m	72,6±2,7	74,3±2,8	p>0,1
LV mass index, g/m^2	131,6±3,6	127,4±3,7	p>0,1
Body mass index, kg/m^2	32,7±1,9	27,2±1,7	p<0,05
Waist circumference, cm	96,8±2,7	88,4±1,5	p<0,05
Triglycerides, mmol/L	2,61±0,11	1,89±0,12	p<0,05
HDL, mmol/L	4,89±0,14	4,26±0,12	p<0,05
HOMA-IR index	2,96±0,09	1,65±0,08	p<0,05

Note: n – number of patients.

Analysis of pulmonary function, patients' complaints, life history and physical examination revealed that all the research participants had the stage II of airflow limitation according to GOLD spirometric classification. It also should be noted that in the group I mixed ventilatory defects (obstructive and restrictive) were verified, whereas patients in the group II had obstructive ventilatory defects (table 2)

Table 2: Basic spirometry parameters in patients with different COPD phenotypes (M±m)

Parameter	Group I (n=48)	Group II (n=39)	Probability value
VC (% out of due)	63,8±3,1	74,4±2,9	p<0,05
FEV1 (% out of due)	62,6±1,74	64,1±1,67	p>0,1
FEV1/FVC (%)	69,6±1,9	68,7±2,2	p>0,1
FEF 25% (% out of due)	46,2±2,3	49,6±2,4	p>0,1
FEF 50% (% out of due)	38,9±1,6	45,5±2,3	p<0,05
FEF 75% (% out of due)	39,6±2,4	47,5±2,9	p<0,05

Note: n – number of patients.

Analysis of table 2 data reveals that patients of group I with chronic bronchitis phenotype also had restrictive ventilatory defects (low vital capacity) due to abdominal obesity as one of the components of metabolic syndrome. Also, patients of group I had significant airway obstruction at the level of medium and small caliber bronchioles that manifested in significantly lower FEF50 and FEF75 ($p<0,01$) than in control group. Severity of these ventilatory defects can be explained as a result of combination of airway obstruction (as manifestation of COPD itself) and indirect influence of "short circuit" phenomenon resulted from abdominal visceral obesity (as manifestation of metabolic syndrome).

Complex analysis of parameters of immune and cytokine status of patients with different COPD phenotypes was also performed (Table 3)

Table 3: Immune and cytokine status of patients with different COPD phenotypes (M±m)

Immunological parameters	Group I (n=48)	Group II (n=39)	Control group (n=36)
$CD54^+$ lymphocytes,%	19,8±1,13	12,2±0,49*	11,07±1,65
$CD11b^+$ lymphocytes,%	35,7±2,1	23,2±1,6*	21,5±1,4
$CD62L^+$ lymphocytes,%	42,6±1,3	30,9±1,6*	28,3±1,7
$CD30^+$ lymphocytes,%	2,03±0,01	0,99±0,01*	1,8±0,03
$CD25^+$ lymphocytes,%	17,2±0,43	21,50±0,47*	12,71±0,39
TNF-α, pg/mL	124,6±6,9	112,7±5,7	42,3±4,9
IL-1β, pg/mL	111,8±5,2	109,6±5,8	39,42±4,5
IL-6, pg/mL	70,4±2,4	42,9±2,9*	10,31±2,3
IL-8, pg/mL	36,5±2,1	37,1±1,75	12,7±1,5
IFN-γ, pg/mL	88,6±3,9	133,9±2,13*	96,4±8,6
IL-4, pg/mL	18,1±0,96	10,7±0,83*	25,42±3,3
IL-10, pg/mL	31,4±2,1	20,7±1,46*	41,75±2,8
TGF-β, pg/mL	123,8±8,12	62,3±7,4*	39,4±4,1
IL-17A, pg/mL	58,1±2,6	20,3±1,1*	17,3±2,7
IL-17 spon., pg/mL	152,5±9,6	95,3±8,2*	97,9±7,5
IL-17 stim., pg/mL	206,7±15,1	170,2±8,5*	164,8±12,3
IL-21, pg/mL	73,7±3,5	41,4±2,8*	38,9±2,2
sVCAM, pg/mL	56,1±2,9	55,3±1,6	18,6±1,9
sICAM-1, ng/mL	419,4±12,7	308,3±12,4*	275,5±17,29

Note: * - probability value ($p<0,05$)
n – number of patients.

Analysis of the data presented in the Table 3 revealed that patients with chronic bronchitis phenotype and evident signs of metabolic syndrome had significantly higher level of activated lymphocytes with different adhesive molecules expression in comparison to healthy persons and patients with emphysema phenotype. Therefore, $CD54^+$ lymphocytes with ICAM-1 expression level was 62,3% ($p<0,05$) higher than that of patients of the group II; $CD11b^+$ lymphocytes with integrin α-chain expression level was 53,9% ($p<0,05$) higher; $CD62L^+$ lymphocytes (L-selectin that provides adhesion of lymphocytes to endothelial wall) level was 37,9% ($p<0,05$) higher. This probably was caused by metabolic syndrome and subclinical atherosclerotic inflammation presented in the patients of the group I that was accompanied by cell adhesion and aggregation changes that occur during atherosclerotic plaque formation.

High percentage of lymphocytes with adhesion molecules expression in group I was combined with high concentration of soluble adhesion molecules in serum. Adhesion molecules play a key role in the immune response and, indeed, in the development of atherosclerotic inflammation since they are expressed on the immune cell surface and bind to their contrreceptors causing cell adhesion and local accumulation, eventually leading to stasis and thrombosis. Among the early markers that reflect the inflammation activity, especially on early stages of disease, there are ICAM-1, VCAM, E-selectin. ICAM-1 is an adhesion molecule from the immunoglobulin family and is expressed on the surface of activated endothelium [1]. High serum concentration of sVCAM was observed in both COPD phenotypes, but was significantly higher than that of healthy persons. At the same time, sICAM-1 concentration was 36,04% ($p<0,05$) higher in the group I than that of group II. Level of these subpopulations was not significantly higher in group II with emphysema phenotype in comparison to healthy persons, and high serum concentration of soluble molecules can be explained by concomitant hypertension. Hence, inflammation of higher activity with higher levels of proinflammatory cytokines leads to more vigorous activation of immunocompetent cells and synthesis of adhesion molecules.

Patients with emphysema phenotype had significantly lower relative number of $CD30^+$ lymphocytes (activated lymphocytes that can conduct a signal for apoptosis, their quantity reflects the level of Th2 cells). $CD30^+$ lymphocytes level was 51,23% lower ($p<0,05$) than that of the group I and $CD25^+$ lymphocytes (Th1 cells) level was 25% higher. Therefore, patients with emphysema phenotype had Th1 predomination of the immune response.

Table 3 shows that the level of proinflammatory cytokines, such as TNF-α, IL-1β and IL-8 was elevated in both groups and did not depend on COPD phenotype. Also the level of IL-6 was higher in both groups in comparison to the control group, but patients with chronic bronchitis phenotype had its concentration 64,1% higher than that in patients with emphysema phenotype.

In the group II of patients with emphysema phenotype significantly higher level of IFN-γ in serum along with lower lever of IL-4 and IL-10 was revealed in comparison to the group I. This is one more evidence of Th1 response predomination in these patients.

At the same time patients with chronic bronchitis phenotype had higher concentration of IL-17A, TGF-β and IL-21 along with significantly lower level of IFN-γ and higher level of IL-4 and IL-10. It demonstrates the predomination of Th-17 immune response in this group. Also in this group of patients higher spontaneous and mitogen-stimulated production of IL-17 (IL-17 spon. and stym.) by the immunocompetent cells was observed. High concentration of IL-17 in serum is a direct evidence of active Th17 response.

Therefore, COPD patients with chronic bronchitis phenotype had higher level of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IL-8, caused by inflammatory processes in the bronchial tree, as well as by systemic inflammation and vascular endothelium damage resulted from atherosclerotic inflammation. It should be also mentioned that high level of IL-6 is a marker of atherosclerotic plaque destabilization and "fatal events" in the coronal arteries [1]. Elevated concentration of TGF-β reflects a key role of this cytokine in progression of pathologic changes in the bronchial tree. TGF-β, as fibrogeneous cytokine, is known to stimulate pathomorphologic changes of the bronchial wall and its remodeling, that eventually leads to development of partially reversible or irreversible bronchial obstruction, that is a basis of COPD pathogenesis [7]. TGF-β in combination with IL-6 is known to induce the differentiation of peripheral blood T-cells into Th17 and enhance the synthesis of IL-17 [7]. This category of patients is also confirmed to have pathologic changes in adhesion processes and cooperation between immunocompetent cells that manifests in their vigorous activation, higher effector abilities and migration to the inflammation site along the vascular wall. Expression of adhesive molecules on endothelial surface and lymphocytes is the first stage of their recruiting to inflammatory site with great variety of following immunologic reactions that essentially have protective nature, but in case of its prolonged persistence or high intensity can lead to dystrophic or necrotic changes. This can be observed during atherosclerotic vascular wall inflammation.

Patients with emphysema phenotype demonstrate signs of Th1 response predomination with high concentration of IFN-γ and proinflammatory cytokines – TNF-α, IL-1β and IL-8 in serum along with significantly lower concentration of IL-4 and IL-10 in comparison to that of the patients with chronic bronchitis phenotype, as well as higher percentage of $CD25^+$ lymphocytes with α-chain of IL-2 receptor expression and lower level of $CD30^+$ lymphocytes. Therefore, patients with emphysema phenotype have Th1 predomination of the immune response.

Conclusions.

1. Patients with chronic obstructive pulmonary disease with chronic bronchitis phenotype were observed to have signs of metabolic syndrome, lipid metabolism disorders and insulin resistance, unlike patients with emphysema phenotype. Patients of both clinical phenotypes have an equal severity of airway obstruction, exacerbation frequency and systemic arterial hypertension as a concomitant disease.
2. Patients with COPD with phenotype of chronic bronchitis and frequent exacerbations have Th17 response predomination with significant elevation of IL-17, TGF-β, IL-6 concentrations in serum and high concentration of soluble adhesion molecules along with high expression of receptors to adhesion molecules on activated lymphocytes of peripheral blood.
3. Patients with COPD emphysema phenotype with frequent exacerbations in immune system have Th1 response predomination with significantly higher concentrations of IFN-γ and lower levels of IL-4 and IL-10, along with high level of activated lymphocytes with $CD25^+$ phenotype and low with $CD30^+$ phenotype.
4. In both groups of patients with chronic obstructive pulmonary disease a high serum level of proinflammatory cytokines (TNF-α, IL-1β and IL-8) was observed.

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