CASPASE-8 AND CASPASE-3 ACTIVITY IN LYMPHOCYTE LYSATE OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND INTESTINAL DYSBIOSIS

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Summary

With the aim to define the role of lymphocyte apoptosis in the mechanisms of T-cell deficiency in patients with chronic obstructive pulmonary disease (COPD) accompanied by the disturbances of intestinal microbiocynosis, the investigation of caspases activity in lysate of lymphocytes was conducted. III degree of dysbiosis was found to be followed by the reduction of $CD3^+$- and $CD8^+$-cells number, and in patients with IV degree of intestinal microbiocynosis the level of lymphocytes of $CD3^+$, $CD4^+$, $CD8^+$ phenotypes decreased in the peripheral blood, followed by the inhibition of phytohaemagglutinin (PHA) induced lymphocyte blast transformation. The activity of caspase-8, caspase-3 and caspase-1 in lymphocyte lysate of patients with COPD was of high rates in dysbiosis of III and IV degree.

Key words: chronic obstructive pulmonary disease, dysbiosis, lymphocytes, apoptosis, immune deficiency

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Introduction

The literary evidences suggest, that presence of intestinal dysbiosis is associated with more severe clinical course of chronic obstructive pulmonary disease (COPD) [1]. Intestinal microbiocynosis disturbances have been postulated to correlate with the disorders of the immune system functions [2].

The results of immunological examinations are indicative of the fact, that the level of $CD3^+$-lymphocytes and lymphocytes proliferative response on T-cell mitogens in lymphocyte blast transformation test with phytohaemagglutinin (PHA) was significantly lower in patients with COPD accompanied by intestinal dysbiosis as compared with clinical groups of patients without concomitant dysbiosis. Moreover, exacerbation of inflammatory process in the bronchi of patients with COPD and dysbiosis was characterized by the decrease of $CD8^+$-lymphocyte blood level, whereas in patients with COPD without concomitant intestinal dysbiosis the level of cells with

$CD3^+CD8^+$ phenotype wasn't changed. The findings obtained might be interpreted by migration of definite clones of T-lymphocytes into *loco morbi*, determining typical for COPD infiltration of the bronchi mainly by $CD8^+$-lymphocytes. However, if in case of COPD without concomitant dysbiosis this migration of definite lymphocyte clones wasn't followed by their corresponding decrease in the peripheral blood, in the presence of dysbiosis this decrease reflects the significant immune imbalance, and evidences marked thymic insufficiency [1].

Materials and Methods

Objective of the research was to determine the role of lymphocyte apoptosis in the development of T-cell insufficiency in patients with COPD accompanied by disturbances of intestinal microbiocynosis. To accomplish this, 98 patients with COPD, aged between 17 and 75 years, – 41 women and 57 men, – were examined. The diagnosis of COPD was made on the basis of analysis of clinical course of the disease and results of spirographic examination (progressing reduction of Tiffeneau-index) [3]. The treatment of COPD was carried out according to the generally accepted approach.

By means of microbiological analysis intestinal normocenosis was diagnosed in 12 patients (group 1), dysbiosis of I degree – in 11 patients (group 2), dysbiosis of II degree – in 15 patients (group 3), dysbiosis of III degree – in 23 patients (group 4), dysbiosis of IV degree – in 37 patients (group 5). 17 healthy individuals, aged from 17 to 70 years, served as a control group.

To assess the state of cellular immunity, lymphocyte subpopulations were identified by $CD3^+$, $CD4^+$ $CD8^+$–determination clusters with usage of the monoclonal antibodies panel to leukocyte differentiated antigens of series LT НПО «Сорбент» (Russia). The samples were examined by means of luminescent microscope «МЛ-2» (Russia). In lymphocyte blast transformation test phytohaemagglutinin (PHA) was used as T-cell mitogen. Caspase-3 and caspase-8 activity was determined in lysate of lymphocytes (10^5/ml), prepared by freezing and thawing method after separation of lymphocytes from the peripheral blood by Ficoll-Verographine-gradient (BioVision, USA) density centrifugation. Statistical analysis of the results was performed by means of «Biostat» software, using Student's t-criterion [4].

Results and Discussion

The level of $CD3^+$-cells in the blood of patients with COPD accompanied by normocenosis and dysbiosis of I and II degrees didn't significantly differ from that of the controls (Table 1). Meantime, in patients with intestinal microbiocynosis disturbances of III and IV degrees the level of T-lymphocytes in the peripheral blood decreased by 12,8 and 27,3 % respectively as compared with that in controls. In patients with COPD and dysbiosis of III degree the level of $CD3^+$-cells was 11,2% lower as compared with that in patients with normocenosis. In case of disorder of the intestinal flora species composition of IV degree the lowest T-lymphocyte blood level was observed, ranking 72,7% of control level and 74,0, 75,3, 80,5 and 83,3% – of those indices in patients with normocenosis, with COPD accompanied by dysbiosis of I, II and III degrees respectively. $CD4^+$-cells content in the blood corresponded to that of the controls in patients of all studied groups except those with dysbiosis of IV degree, who demonstrated the lowering of $CD4^+$-cells level by 27,8% concerning that in controls and by 26,5, 26,4, 26,5 and 19,6% – as compared with corresponding indices in patients with COPD accompanied by normocenosis and dysbiosis of I, II and III degrees respectively. The number of $CD8^+$-cells in the peripheral blood wasn't significantly changed in patients with normocenosis and those with COPD accompanied by

dysbiosis of I and II degrees, being considerably reduced in patients with disorder of the intestinal flora species composition of III and IV degrees. $CD8^+$-cells level in patients with COPD and dysbiosis of III degree was by 23,2% lower than in control individuals and by 23,3, 19,0 and 17,4% lower as compared with that index in patients with normocenosis and dysbiosis of I and II degrees. The lowest level of $CD8^+$-cells was observed in the blood of patients with dysbiosis of IV degree, ranking only 66,9% of control level and 66,9, 70,6, 72,0 and 87,2% – of that in patients with normocenosis and those with COPD accompanied by dysbiosis of I, II and III degrees respectively. Despite a decreased level of $CD4^+$- as well as $CD8^+$-cells, immunoregulatory index wasn't significantly changed in any of the studied groups of patients. These findings obtained are proved by existing literature data [1].

Table 1. **Level of T-lymphocytes and their subclasses in the blood of patients with COPD accompanied by dysbiosis of various degrees**

Groups of patients	$CD3^+$, %	$CD4^+$, %	$CD8^+$, %	$CD4^+/CD8^+$
Controls (n=17)	67,5±2,16	46,1±1,96	22,8±1,49	2,1±0,15
Patients with COPD and normocenosis (n=12) – *group 1*	66,3±2,71	45,3±2,63	22,8±1,79	2,1±0,20
Patients with COPD and dysbiosis of I degree (n=11) – *group 2*	65,2±3,16	45,3±3,03	21,6±1,24	2,2±0,21
Patients with COPD and dysbiosis of II degree (n=15) – *group 3*	60,9±2,69	45,3±2,65	21,2±1,15	2,3±0,21
Patients with COPD and dysbiosis of III degree (n=23) – *group 4*	58,9±2,16 P<0,01 P_1<0,05	41,4±1,94	17,5±0,96 P<0,01 P_1<0,01 P_2<0,02 P_3=0,02	2,6±0,19
Patients with COPD and dysbiosis of IV degree (n=37) – *group 5*	49,1±1,58 P<0,001 P_1<0,001 P_2<0,001 P_3<0,001 P_4<0,001	33,3±1,75 P<0,001 P_1<0,01 P_2<0,01 P_3<0,001 P_4<0,01	15,3±0,59 P<0,001 P_1<0,001 P_2<0,001 P_3<0,001 P_4<0,05	2,3±0,16

Note: values are expressed as means ± standard errors;

P – significant difference in comparison with control (P≤0,05);

P_1 – significant difference in comparison with patients of group 1 (P≤0,05);

P_2 – significant difference in comparison with patients of group 2 (P≤0,05);

P_3 – significant difference in comparison with patients of group 3 (P≤0,05);

P_4 – significant difference in comparison with patients of group 4 (P≤0,05);

n – number of patients in a group.

To find the mechanisms of defined abatement of cellular immunity in patients with COPD accompanied by dysbiosis the study of lymphocyte apoptosis and its effectors – caspases (caspase-3 and caspase-8 particularly) was carried out. Thus, caspase-1 is known to translate the inducing signal for the production of cytokines, improving the functional activity of phagocyte blood cells [5]. The results of examination of caspases activity in lymphocyte lysate (10^5cells/ml) are presented in *Table 2*. Caspase-8 activity in lymphocyte lysate of patients with COPD and normocenosis as well as those with dysbiosis of I and II degrees didn't significantly differ from that in controls. In patients with COPD accompanied by dysbiosis of III degree caspase-8 activity increased by 41,5% concerning controls, in patients with dysbiosis of IV degree – by 95,7%. In case of disorder of the intestinal flora species composition of III degree the activity of caspase-8 was higher, than in case of normocenosis and dysbiosis of I degree by 37,3 and 30,5% respectively, being, however, not

significantly different as compared with those indices in patients with dysbiosis of II degree. In the lysate of peripheral blood lymphocytes of patients with COPD accompanied by dysbiosis of IV degree the activity of caspase-8 was found to be the highest, exceeding that of the patients with normocenosis and dysbiosis of I, II and III degrees by 89,9, 80,5, 62,2 and 38,3% respectively.

Table 2. **Caspase activity in lymphocyte lysate of COPD patients with dysbiosis of various degrees**

Groups of patients	Caspase-8, UE/ml	Caspase-3, UE/ml	Caspase-1, UE/ml
Controls (n=17)	1,01±0,092	0,81±0,078	0,50±0,054
Patients with COPD and normocenosis (n=12) – *group 1*	1,04±0,151	0,99±0,132	0,72±0,109
Patients with COPD and dysbiosis of I degree (n=11) – *group 2*	1,09±0,129	0,90±0,120	0,79±0,119 P<0,02
Patients with COPD and dysbiosis of II degree (n=15) – *group 3*	1,21±0,115	1,05±0,109	0,89±0,103 P<0,01
Patients with COPD and dysbiosis of III degree (n=23) – *group 4*	1,42±0,094 P<0,01 P_1<0,05 P_2=0,05	1,26±0,091 P<0,001 P_2<0,05	1,11±0,086 P<0,001 P_1=0,01 P_2<0,05
Patients with COPD and dysbiosis of IV degree (n=37) – *group 5*	1,97±0,097 P<0,001 P_1<0,001 P_2<0,001 P_3<0,001 P_4<0,001	1,90±0,088 P<0,001 P_1<0,001 P_2<0,001 P_3<0,001 P_4<0,001	1,68±0,080 P<0,001 P_1<0,001 P_2<0,001 P_3<0,001 P_4<0,001

Note: values are expressed as means ± standard errors;
P – significant difference in comparison with control (P≤0,05);
P_1 – significant difference in comparison with patients of group 1 (P≤0,05);
P_2 – significant difference in comparison with patients of group 2 (P≤0,05);
P_3 – significant difference in comparison with patients of group 3 (P≤0,05);
P_4 – significant difference in comparison with patients of group 4 (P≤0,05);
n – number of patients in a group.

No statistically significant difference was noted concerning the activity of caspase-3 in patients with COPD accompanied by normocenosis as well as by dysbiosis of I and II degrees as compared with controls. The activity of this enzyme in case of dysbiosis of III degree was elevated by 55,2% concerning that of controls, and in case of dysbiosis of IV degree caspase-3 activity ranked the highest level, being 2,3 times higher than found in control individuals. In case of dysbiosis of III degree caspase-3 activity exceeded that of patients with dysbiosis of I degree by 39,7%, but wasn't significantly different from that of patients with COPD and dysbiosis of II degree. At the same time, in patients with maximal degree of disorder of the intestinal flora species composition the activity of caspase-3 was 1,9, 2,1, 1,8 and 1,5 times higher, than in those with COPD accompanied by normocenosis and dysbiosis of I, II and III degrees respectively. The increase of caspase-1 activity in patients with normocenosis wasn't statistically significant as compared with that in controls, whereas in patients with COPD accompanied by disorder of the intestinal flora species composition this index substantially exceeded control rates: by 58,0% – in case of dysbiosis of I degree, by 76,5% – in dysbiosis of I degree, by 2,2 and 3,3 times – in dysbiosis of III and IV degrees respectively. Caspase-1 activity in patients with COPD and dysbiosis of III degree was higher as compared with that in patients with normal intestinal microbiocenosis and with dysbiotic disorders of I degree (by 54,0 and 40,2% respectively), but wasn't significantly changed as compared with caspase-1 activity in patients with dysbiosis of II degree. Caspase-1 activity of in case of disorder of

the intestinal flora species composition of IV degree markedly exceeded that in patients of all studied groups: by 2,3 times as compared with patients with normocenosis, by 2,1 times – with patients with dysbiosis of I degree, by 89,6 and 51,5% – with dysbiosis of II and III degrees respectively. Thus, the activity of caspase-8, caspase-3 and caspase-1 in lymphocyte lysate of patients with COPD gained high rates in dysbiosis of III and IV degree, although being not precisely directly proportional to the degree of disorders of the intestinal flora species composition.

According to modern views, apoptosis is one of the fundamental forms of cell reaction on external and internal signals, alternative to proliferation and differentiation. The regularity of biochemical reactions – cascade activation of caspases – is now considered to be key-point for apoptosis. Progression of apoptosis is controlled by the products of proto-oncogenes, either stimulating caspases activation, or blocking it. The most studied is Bcl-2, which homodimeric form inhibits apoptosis, whereas heterodimer Bax/Bcl-2 activates it, and Bcl-x, existing in two forms (due to alternative splicing) – a "long" one, Bcl-xi, inhibiting apoptosis, and a "short" one, Bc1-xs, stimulating it. There are three pathways of apoptosis induction. The first one is carried by means of specific Fas (CD95) – receptors and tumor necrosis factor receptor – TNFRI (CD120a) with further activation of caspase-8 (Fas) and caspase-2 (THFRI). The other pathway is also initiated through receptors, but in case of deficient or absent signaling. Thus, young representatives of practically all hemopoietic lines require external signals for their survival, and cytokines are their source. In case of lymphocyte activation the presence of antigen without co-stimulating signals, coming through CD28 to T-cells and through CD40 to B-cells, results in their anergy or apoptosis. Besides, lymphocyte activation under conditions of growth factors deficiency (IL-2 for T-cells, IL-4 for B-cells) is completed with apoptosis, as well as in case of repeated action of growth factor upon the preliminary activated cells. The third mechanism is connected with an inner signal inducing apoptosis (accumulation of non-repaired ruptures of DNA or its modification), realized by the involvement of the nuclear protein p53 [6, 7]. Lymphocyte apoptosis in patients with COPD accompanied by dysbiosis is likely to occur by means of the first or second pathways, since disorders of intestinal microbiocynosis influence upon the factors of immune response regulation considerably [1, 8, 9, 10].

Thus, COPD patients with normocenosis and dysbiosis of I-II degrees don't demonstrate any changes of $CD3^+$-, $CD4^+$- and $CD8^+$-lymphocytes content in the blood. In case of dysbiosis of III degree decrease of $CD3^+$- and $CD8^+$-cells is found, and in patients with disorders of intestinal microbiocynosis of IV degree lymphocyte level of all three phenotypes – $CD3^+$, $CD4^+$, $CD8^+$ – decreases in the peripheral blood, followed by inhibition of PHA-induced lymphocyte blast transformation reaction. The activity of caspase-8, caspase-3 and caspase-1 in lymphocyte lysate of COPD patients is found to be high in case of dysbiosis of III and IV degrees.

References

1. Білоглазов ВО. Роль дизбактеріозу і цитокінів слизової оболонки кишківника в формуванні системного і регіонального імунного дизбалансу у хворих на бронхіальну астму і хронічний обструктивний бронхіт. Актуальні проблеми клінічної імунології та алергології 1998; 3:32-40.
2. Дранник ГН. Клиническая иммунология и аллергология. Одесса: Астро Принт, 1999:98-102.
3. Гриппи МА. Патофизиология легких. М.: Бином, 1997.
4. Гланц С. Медико-биологическая статистика. М.: Практика, 1999.
5. Владимирская ЕБ. Механизмы апоптотической смерти клеток. Гематология и трансфузиология 2002; 47(2):35-40.

6. Петухов ВИ. Роль Fas-опосредованного апоптоза в реализации противоопухолевого эффекта α-интерферона при хроническом миелолейкозе. Гематология и трансфузиология 2000; 45(4):29-33.
7. Скворцова ВИ. Участие апоптоза в формировании инфаркта мезга. Инсульт 2001; 2:12-18.
8. Варбанец ЛД. Эндотоксины грамотрицательных бактерий: структура и биологическая роль. Мікробіол. ж. 1994; 56(3):76-97.
9. Возианов АФ, Бутенко АК, Зак КП. Цитокины. Биологические и противоопухолевые свойства. К.: Наукова думка, 1998.
10. Воложин АИ. Дизрегуляция иммунологических механизмов и фагоцитарной активности лейкоцитов – ведущая причина острого воспаления. Патол. физиология и эксперим. терапия 1997; 2:29-31.