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FEATURES OF HUMORAL REACTIONS FORMATION IN PATIENTS WITH COMBINED COURSE OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND CHRONIC PANCREATITIS

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Abstract

The article highlights features of humoral immune response formation in isolated course of chronic obstructive pulmonary disease and in its combination with chronic pancreatitis.

The assessment of changes in indicators of the complement system (total activity, components C3 and C5), immunoglobulin profile, and content of circulating immune complexes in patients with isolated disease and in case of comorbidity was done. In patients with the isolated chronic obstructive pulmonary disease we observed changes in concentrations of immunoglobulins of the main classes: reduction of immunoglobulins M and A against the increase of immunoglobulins G and E. At the same time, there was a decrease of the total complement activity and concentration of its fragments at the high content of circulating immune complexes. In case of the chronic obstructive pulmonary disease and chronic pancreatitis comorbidity there was a redistribution of emphasis on indicators of humoral immunity manifested by the increased content of circulating immune complexes and immunoglobulins of all classes, with simultaneous hyperactivation of the complement system. Such deviations in humoral response formation in terms of comorbidity might initiate autoimmune reactions development and accumulation of antibodies to target organs with the subsequent formation of relevant complications.

Thus, the presence of concomitant chronic pancreatitis exacerbated changes in rates of nonspecific immunity, which might be associated with a high risk of complications in mentioned patients group and is an unfavorable prognostic factor.

Keywords: *chronic obstructive pulmonary disease, chronic pancreatitis, complement system, immunoglobulins, circulating immune complexes.*

Introduction

Nowadays the role of the immune system in development of both acute and chronic inflammatory processes is proven and undeniable. It is generally admitted that any chronic disease leads to excessive load on the immune system, and, ultimately, sooner or later, induces formation of secondary immunodeficiency and reduces the effectiveness of immunological response reactions of the organism [*Male D. et al., 2007; Drannik G., 2010; Shishido S. et al., 2012*].

The immune response is realized through the development of both cellular and humoral reactions, which, although differentiated into separate groups, are actually interrelated links of a single process. Depending on the type of antigen, the way it enters the body, duration, and multiplicity of pathogenic actions this process initializes certain cellular elements and directly involves specific antibodies and other factors of humoral immunity [*Male D. et al., 2007; Drannik G., 2010; Shishido S. et al., 2012*].

The humoral factors – a variety of cytokines, immunoglobulins, components of the complement system and a number of others – provide the interaction of immunocompetent cell elements both circulating in body fluids (lymphocytes, monocytes, eosinophils, neutrophils, etc.) and fixed ones (epitheliocytes, endotheliocytes, fibroblasts, etc.) [*Male D. et al., 2007; Drannik G., 2010; Trouw L., Daha M.,*

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2010; Daha N. et al., 2011; Shishido S. et al., 2012]. The factors of nonspecific humoral immunity implement the so-called "acute phase response" of the organism through a number of proteins – C-reactive protein, fibrinogen, haptoglobin, ceruloplasmin and other acute phase reactants [*Pepys M., Hirschfield G., 2003; Verma S. et al., 2004; Gruys E., Toussaint M., 2005; Shishido S. et al., 2012*].

In this case, one of the leading roles is played by the group of thermolabile plasma proteins representing complement system, the activation of which leads to lysis of the antigens cell membrane [*Trouw L., Daha M., 2010; Carroll M., Sim R., 2011; Ehrnthaller C. et al., 2011*]. This system is one of the main protein systems of innate immunity. Among its most significant biological effects one should mention the ability to increase capillary permeability, stimulation of phagocytosis and chemotaxis, activation of granulocytes and macrophages, opsonization, cytolysis, immune adherence, release of biologically active amines (histamine, bradykinin, serotonin) from the granules of tissue basophiles, destruction of viruses and virus-affected cells, and a number of other important phenomena [*Chen M. et al., 2010; Drannik G., 2010; Carroll M., Sim R., 2011; Ehrnthaller C. et al., 2011*].

The activation of complement system occurs mainly through the classical pathway – by type of the enzymatic reaction cascade – resulting in formation of a number of soluble and insoluble enzymes and complexes, which are eventually responsible for certain biological effects. The classical pathway of complement system activation begins with its first component – C1 – and is followed with subsequent involvement of the central link of the system – C3 fragment – and supposes a direct participation of immunoglobulins M (IgM) and G (IgG) in the process performing the antibody function in the organism and taking a part in the antigen-antibody complex [*Male D. et al., 2007; Drannik G., 2010; Carroll M., Sim R., 2011; Ehrnthaller C. et al., 2011*]. This latter explains the fact that the complement is not activated and does not interact with free circulating antibodies, as the antibody binding site of the C1 component becomes available only after its binding to an antigen and the immune antigen-antibody complex formation [*Chen M. et al., 2010; Trouw L., Daha M., 2010; Carroll M., Sim R., 2011; Daha N. et al., 2011; Ehrnthaller C. et al., 2011*].

Besides the classical pathway of complement activation, there is an alternative one that is implemented without the involvement of immune complexes, as in this case the process starts with the direct activation of C3 component and, in contrast to the classical pathway, does not require C1, C2 and C4 fragments involvement. In this situation, a necessary condition is participation of properdin system proteins – properdin, D and B factors – indeed these compounds play the role of C3 component activators in this case [*Male D. et al., 2007; Chen M. et al., 2010; Drannik G., 2010; Trouw L., Daha M., 2010; Daha N. et al., 2011; Ehrnthaller C. et al., 2011*].

Along with the above described classical and alternative pathways of complement activation, there is also a third route associated with lectin that activates complement components in the same sequence as in case of the classical pathway, but the presence of antibodies is not a necessary condition [*Male D. et al., 2007; Chen M. et al., 2010; Drannik G., 2010; Trouw L., Daha M., 2010; Daha N. et al., 2011*]. The role of lectin pathways activators can be performed by a number of Gram-positive and Gram-negative bacteria, fungi, mycobacteria and other infectious agents, the surfaces of which contain mannose residues that can interact with mannose-binding lectin of the blood serum. As a result of this interaction the protein acquires ability to bind to C1 fragment and thus triggers the complement system on the classical pathway without participation of immune complexes [*Male D. et al., 2007; Chen M. et al., 2010; Drannik G., 2010; Trouw L., Daha M., 2010; Daha N. et al., 2011*].

As a result of the complement system activation in this or that way, it eventually leads to the membrane attack complex formation that once absorbed in a lipid bilayer of target cells destroys the integrity of the cell membrane, which, in its turn, leads to distortion of the ion, water, and amino acid transmembrane exchange and finally to osmotic death of the cell [*Male D. et al., 2007; Chen M. et al., 2010; Drannik G., 2010; Carroll M., Sim R., 2011; Daha N. et al., 2011*].

Despite the fact that the alternative pathway of complement activation is the immediate reaction of an organism to the antigen introduction, as in this case no time is required for formation of the antigen-antibody complex and activation of the first components of the complement system, the

main pathway of its activation, particularly in the conditions of chronic pathology, is the classic one, which is realized with the direct participation of immunoglobulins.

Immunoglobulins are multifunctional proteins, and besides the activation of complement system, they realize other important biological effects in the organism: interaction with other immunocompetent cells and specific recognition of different antigens and haptens, which also play a significant role in the organism immune response development [*Male D. et al., 2007; Drannik G., 2010*].

Thus, the key role in the realization of inflammatory reactions belongs to humoral factors of natural immunity, which are formed in accordance with the general laws of the immune response. At the same time, comorbid course of the internal organs diseases can make some adjustments to the sequence and severity of these reactions, which, in their turn, can be manifested by increased clinical symptoms, more rapid progression of the process and formation of early complications.

The aim of the present study was to estimate the state of nonspecific immunity in comorbidity of chronic obstructive pulmonary disease (COPD) and chronic pancreatitis (CP).

MATERIAL AND METHODS

Ninety-seven patients with COPD were under monitoring; chronic pancreatitis was diagnosed in 59 subjects, and they composed the main group. The average age of patients with comorbidity was 44.2±5.7 years, with a male-dominated group (43 persons: 72.9%). A comparison group involved 38 patients with isolated COPD and the average age of 43.9±4.9 years; male subjects were in the majority (27 patients: 71.1%).

Indicators of norm were obtained after examination of 20 healthy patients, who were representative by gender and age, providing a basis for comparison.

Concentrations of immunoglobulins of different classes – A, M, G – were determined using Mancini method of radial immunodiffusion in gel. Calculation in the peripheral blood was performed comparing the obtained radius with the standard logarithmic scale. In determining the immunoglobulin E (IgE) we used method of sedimentation by rivanol.

The state of complement system was assessed by determining the content of its main components, C3 and C5, and the total amount of complement using the method of 50% hemolysis with recalculation by a standard schedule. Circulating immune complexes (CICs) in serum of patients were determined by a method based on the ability of polyethylene glycol with a molecular weight of 6000 *Da* at low concentrations (3.5%) to precipitate immune complexes. The subsequent measurement of the solution optical density was carried out using "SF-26" spectrophotometer (Russia) at a wavelength of 280 *nm* and data expressed in conditional units.

Statistical analysis of the results was performed using PC with licensed programs "Microsoft Excel and "Statistica 6.0". In this case, we calculated the mean values (M) and their errors (m) applying non-parametric statistical methods (Mann-Whitney test). Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

In patients with the isolated course of COPD levels of IgG and IgE were 1.5 and 2.1 times elevated, as compared to the values of healthy individuals, respectively (Table 1). At the same time, we determined a significant reduction of IgM to 2.1 times and IgA to 2.8 times, as compared to the control group. Simultaneously, the level of CICs was detected to be elevated 2.7 times.

TABLE 1.

Content of immunoglobulins main classes and circulating immune complexes in patients with COPD

Parameters	Patients with COPD (n=38)	Healthy persons (n=20)
IgM, *g/l*	0.58±0.2*	1.2±0.11
IgA, *g/l*	1.14±0.13*	3.18±0.12
IgG, *g/l*	18.41±0.47*	12.3±0.1
IgE, *g/l*	2.48±0.29*	1.2±0.1
CICs, *absorbance units*	0.32±0.04*	0.12±0.07

NOTE: * *significant differences in relation to indicators of healthy persons ($p<0.05$).*

Upon the investigation of immunoglobulins in the serum of main group patients – with the combined course of COPD and CP – the content of IgM was found to be significantly increased (2 times) compared to the values of healthy persons (Table 2).

Table 2

Content of immunoglobulins main classes and circulating immune complexes in patients with COPD and CP

Parameters	Patients with COPD and CP (n=59)	Healthy persons (n=20)
Ig M, g/l	2.34±0.23*	1.2±0.11
Ig A, g/l	4.15±0.34*	3.18±0.12
Ig G, g/l	17.71±0.52*	12.3±0.1
Ig E, g/l	2.35±0.22*	1.2±0.1
CIC, absorbance units	0.46±0.02*	0.12±0.07

Note: * - *significant differences in relation to indicators of healthy persons (p <0.05)*

As obvious from data of Table 2, the levels of IgA, IgG and IgE in the peripheral blood of patients with comorbid pathology were respectively 1.3, 1.4 and 2 times higher than the values of healthy persons. The content of CICs was also increased 3.8 times.

The comparison of changes directionality in the classes of immunoglobulins allowed to reveal the following features. In patients with isolated COPD the level of IgA was significantly below control values; apparently, this can be explained by its increased consumption in conditions of the chronic inflammatory process in the bronchopulmonary system, the exacerbation of which was caused by the infectious agent.

In contrast to the isolated COPD, in patients with comorbid pathology we registered elevated levels of IgA. In our opinion, these changes can be explained by an additional activation of the immune system resulting from the involvement in pathological process of pancreas, which is thus trying to create a "line of defense" in response to the infectious agent appearance in the organism.

One of the functions of IgA is activation of the complement system by alternative pathways and protection of the mucous membrane organ from antigens, which are in contact with it [*Male D. et al., 2007; Drannik G., 2010*]. That is, the increase of IgA in patients with combined course of COPD and CP provides two-way initiation of the complement system: both by classical and alternative pathways; this latter is manifested by the excessive total activity of complement and its key components, C3 and C5, and can lead to involvement of target organs into the pathological process.

A similar multidirectionality was diagnosed in the study of IgM content. Patients with a combination of COPD and CP were found to have increased content of IgM, which might be regarded as the result of autoimmune processes. The reduction of IgM concentration in patients with isolated COPD is obviously related to its active use in the CICs formation.

The registered increase of IgG in both groups of patients signified to the presence of chronic inflammation and the active participation of this class of immunoglobulins in regulation of the immune response. Indeed, precisely IgG, besides the active involvement in the immune response, has a regulatory function – by type of the feedback it influences on the activity of other immune mechanisms (humoral and cellular) that provides completeness of the immune response [*Male D. et al., 2007; Drannik G., 2010*]. In this case, the simultaneous increase of IgE levels in both groups on the background of increased IgG content can be interpreted as a high probability of autosensibilization processes development under conditions of chronic inflammation.

In the study on blood serum complement activity and its key components, C3 and C5, it was found that in a comparison group of patients its total activity decreased 1.2 times, as compared with controls (Table 3). Levels of complement components C3 and C5 were established to be reduced compared to values of healthy persons 1.5 and 1.2 times, respectively.

At the same time, in patients of the main group – the combined course of COPD and CP – the total

Table 3

Indicators of complement activity and some of its fragments in patients with COPD

Parameters	Patients with COPD (n=38)	Healthy persons (n=20)
Complement (total activity), U/l	64.1±3.4*	75.4±3.8
Complement (C3), g/l	0.96±0.02*	1.4±0.04
Complement (C5), g/l	53.4±2.87*	64.7±3.2

Note: * - *significant differences in relation to indicators of healthy persons (p <0.05).*

complement activity was determined to be increased 1.2 times, which was caused by the significant increase in the content of C3 and C5 fragments of complement (Table 4).

TABLE 4

Indicators of complement activity and some of its fragments in patients with COPD and CP

Parameters	Patients with COPD and CP (n=59)	Healthy persons (n=20)
Complement (total activity), U/l	87.2±2.65*	75.4±3.8
Complement (C3), g/l	2.95±0.13*	1.4±0.04
Complement (C5), g/l	75.7±2.32*	64.7±3.2

NOTE: * - *significant differences in relation to indicators of healthy persons (p <0.05).*

At the comparative analysis of data it was revealed that the indices of total complement activity in patients of comparison group were 1.4 times reduced in relation to values of the main group ($p<0.05$). At the same time, patients with comorbidity of COPD and CP were determined to have elevated levels of C3 and C5 complement fragments in peripheral blood, and these values were 3.1 and 1.4 times higher than in patients with isolated COPD, respectively.

The decrease in total activity of the complement system in patients with COPD might be caused by the inhibition of its C3 and C5 components production that might occur due to a significant consumption of these plasma proteins in development of inflammatory reactions in chronic infections. Another possible reason for this phenomenon might be violation of formation and/or functional deficiency of macrophages, which are the main producers of components of the complement system in inflammatory focus [*Male D. et al., 2007; Drannik G., 2010*].

At the same time, in patients with COPD and CP hyperactivation of the complement system was observed that was probably the result of the autosensibilization process development. Apparently, in comorbidity the activation of complement system was carried out not only by the classical, but also by the alternative pathway with the primary direct activation of C3. In this case bacterial substances and viruses, which ensure its launch, passing the first five components can be considered as triggers [*Male D. et al., 2007; Drannik G., 2010*]. Such bilateral pathway of the complement system activation is typical for chronic infection, especially in conditions of autoimmune processes domination.

Thus, the combined course of COPD and CP is associated with significant disturbances in the immune system that causes significant activation of nonspecific humoral immunity and development of autosensibilization. Both of two factors can be considered as predictors of disease progression and formation of complications.

CONCLUSION

Active inflammation in the bronchopulmonary system of patients with COPD is accompanied by deviations of humoral immunity indices, which is manifested by changes in concentrations of main immunoglobulin classes: reduction of IgM and IgA against the increase of IgG and IgE. Simultaneously, there is a decrease of the total complement activity and concentration of its fragments, with the elevated levels of CICs.

In conditions of comorbidity (a combination of COPD and CP) the directionality of humoral immunity indicators has its own features: activation of the complement system, elevation in levels of main immunoglobulins classes and CICs.

Such changes might initiate development of autoimmune processes and accumulation of antibodies to target organs with subsequent formation of the relevant complications; all this allow considering the given pathological tandem as prognostically unfavorable.

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