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INVESTIGATION OF AUTOANTIBODIES DIRECTED AGAINST TISSUE-SPECIFIC MYOCARDIAL ANTIGENS IN DILATED CARDIOMYOPATHY

The autoantibodies directed against actin and myosine have been detected in the blood sera of patients with dilated cardiomyopathy (DCMP) at the level that was sufficiently higher than in healthy donors. While comparing of the immunogeneicity of these proteins we have stated that myosine from affected by DCMP myocardium have been more immunogenic then from normal one. In the case of actin the immunogeneicity have been higher for the protein from normal myocard. The investigations of actin by limited proteolysis may suggest that the differencies in immune properties are not connected with primary structure but with structures of higher levels.

Introduction. Nowadays the presence of autoimmune processes during the development of various pathologies including heart diseases is obvious. At the same time the functional activity of autoAbs wasn't examined enough. The investigation of structural and functional properties of autoantibodies (aAbs) directed against antigens from heart and vessels, the establishment of their role while the origin and development of heart diseases is necessary not only for theoretical but for practical aims also, in the context of developing of novel therapeutic and diagnostic preparations [1].

The aim of our investigation was to study the molecular mechanisms of autoantibodygenesis directed against the most important tissue-specific antigens — actin and myosin, during development of dilated cardiomyopathy (DCMP). In recent studies the aAbs directed against adenine nucleotide translocator (ANT) [2], the mitochondrial enzyme from myocytes were detected; the aAbs directed against another mitochondrial enzyme, branched chain keto acid dehydrogenase (BCKD) [3] were also described at DCMP and myocarditis. The laboratory of Limas (USA) during recent years studied the structure and properties of aAbs directed against β-adrenoreceptor [4—8]; these aAbs were detected in 30—40 % of patients with DCMP. The presence of aAbs directed against myosin, tropomyosin, vimentin, laminin at some heart diseases such as postmyocardial infarction syndrome, dilated cardiomyopathy, infectious myocarditis, and doxorubicin (Adriamycin) cardiotoxicity [9] is occasionally accompanied by the deposition of antibodies within the myocardium, particularly on the sarcolemma.

The present paper is a first stage of study of aAbs, their properties and role at DCMP progressing, which are directed against the most abundant heart antigens — actin and myosin.

The method described [10] for purifing of preparative quantities of antigens from human myocardium (pathomorphological material) enabled us to obtain the mentioned above antigens as from normal myocardium as from myocardium, affected by DCMP.

The presence of aAbs in the sera of patients with DCMP was not unexpected because many facts suggested the presence of cellular and humoral immunity in the development of DCMP [4]. These data concern

the introduction of Igs (in the form of immune complexes) in affected myocardium, the abnormalities in relative proportion and function of peripheric lymphocytes, the occurrence of cytokines and existance of various aAbs directed against surface and intracellular components of myocytes. Meanwhile the correlation of mentioned processes with the clinical manifestation of diseases wasn't identified yet.

Materials and methods. The sera from DCMP patients were kindly provided by Strazesko Institute for Cardiology. The 45 sera from patients with DCMP and 38 sera from healthy donors were examined. The

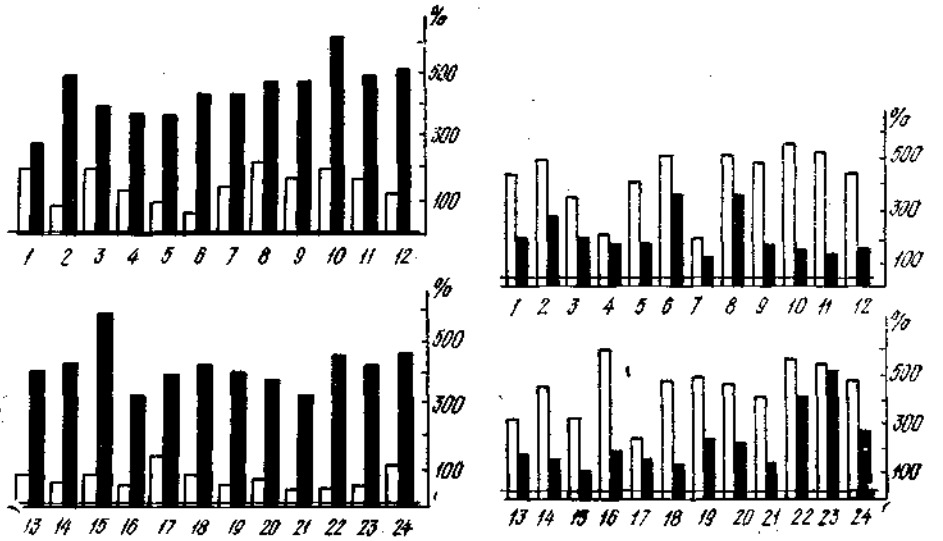

Fig. 1. The immunoreactivity of sera of patients with DCMP directed against myosin purified from normal (light columns) and DCMP myocardium (dark columns). The immunoreactivity was detected as described in Materials and methods
Fig. 2. The immunoreactivity of sera of patients with DCMP directed against actin purified from normal (light columns) and DCMP myocardium (dark columns). The immunoreactivity was detected as described in Materials and methods

diagnosis was provided according to the World Health Organization criteria.

Purification of actin and myosin. The preparations of actin and myosin (90 % purity) were obtained from healthy and affected by DCMP myocardium as described [1]. The purity of preparations was controlled by the electrophoresis according Laemmly [11].

ELISA. The immunoreactivity of the patients sera was detected by the ELISA method [13] with modifications. The antigens were immobilized overnight at 4 °C in 0.1 M carbonate buffer, pH 8.0. The concentrations of antigens were 5 μg per ml. Tubulin, DNA from salmon sperm, trinitrophenol (TNP, «Sigma») and total preparation of human IgG from healthy donors obtained by method [12] were used as control antigens. The human anti-IgG conjugated with peroxidase was purchased from DiaGen, Moscow. The results of ELISA were calculated as described in [14].

Detection of IgG and IgM in the sera of patients. The concentrations of IgG and IgM in the sera investigated were determined by ELISA method [13] with standard human sera from «Boehringer» (Germany) as control.

Limited proteolysis. The limited proteolysis of actin preparations from normal and affected by DCMP myocardium was provided as described [15] with several modifications: pH 8.0, temperature 20 °C, enzyme-substrate ratio 1 : 100, TPCK-treated trypsin from «Sigma» (USA). The proteolysis time was from 15 to 60 min. The results of hydrolysis were detected by SDS-gel electrophoresis [11]. The gels were scanned by Ultroscan (LKB, Sweden).

Results and discussion. Table shows the concentrations of IgG and IgM in the investigated sera of patients with DCMP and healthy donors. It is obvious that Ig concentrations are different and don't depend on kind and stage of disease.

The immunoreactivity of sera from DCMP patients against actin and myosin from normal and affected by DCMP myocardium. Fig. 1 shows the immunoreactivity of DCMP patients sera against myosin from normal and affected by DCMP myocardium. It's obvious that immunoreactivity of sera against DCMP-myosin is higher then in the case of myosin from normal heart. In the case of actin it was vice versa (Fig. 2). As it was shown by us earlier [10] myosin and actin preparations from normal and

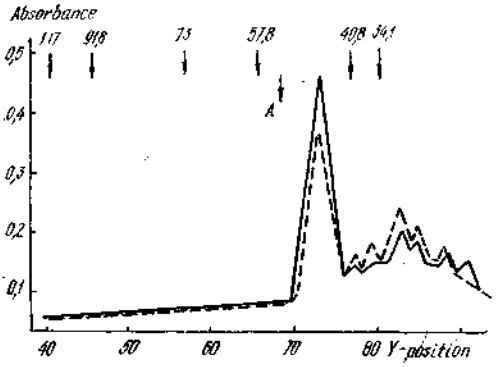

Fig. 3. The densitogramm of proteolytic cleavage of myosine preparations from normal (solid line) and DCMP myocardium (dotted line). The position of molecular mass markers are indicated by arrows. *A* — position of undigested actin. The proteolysis was conducted as described in Materials and methods

DCMP-myocardium possess the same M_r and isoelectric point these results suggest that the structure peculiarities aren't connected with the primary structure of the molecules. We believe that the differencies in the immunogeneicity are connected with certain conformational changes. It's well known that protein folding is sufficient for their immunological properties [16, 17]. The protein conformation depends on posttranslation modifications and intracellular media, that is especially sufficient for actin and myosin molecules, that form «molecular motor» among and with a help of many other proteins: besides tropomyosin and family of troponines, nowadays at least 8 proteins are known to take part in functioning of this system [18].

We proceeded our work in the field of search of possible reasons in the changes of immunogeneicity of myocardial tissue-specific antigens at DCMP progressing on the conformational level of mentioned proteins.

Concentrations of IgG and IgM in the sera of patients with DCMP (1—22) and sera of healthy donors (23—32) (the most immunoreactive sera are presented); 33 — control serum

Serum N	IgM, mg/ml	IgG, mg/ml	Serum N	IgM, mg/ml	IgG, mg/ml
1	1.18	26.43	18	0.9	13.5
2	3,6	23.47	19	0.92	13.7
3	1.7	30.35	20	1.33	14.65
4	3.02	14.63	21	2.29	18.61
5	2.48	14.12	22	0.98	20.48
6	1.9	7.7	23	1.33	19.92
7	1.2	18.77	24	1.1	22.53
8	0.9	14.82	25	2.1	18.17
9	0.5	14.5	26	0.94	15.17
10	0.8	14.08	27	2.23	17.07
11	1.97	11.2	28	0.5	15 22
12	0.95	19.5	29	1.26	16.56
13	1.02	13.2	30	0.96	13.89
14	0.9	26.42	31	2.01	11.2
15	0.95	6.7	32	1.05	23.5
16	1.3	19.5	33	0.58	12.24
17	2.23	25.6			

The first stage of these investigations was the study of the limited proteolysis of actin molecule.

The results of actin limited proteolysis are shown at Fig. 3. From the densitogramm it's obvious that the spectrum of the fragments obtained is alike for both preparations. At the same time the intensivity of digestion of actin from myocardium affected by DCMP is higher then in the case of normal tissue that is expressed in higher concentrations of low molecular weight fragments (14 and 23 % for fragment with M_r 12.000, respectively). The mentioned differencies in the intensivity of actin preparations digestion may suggest the presence of certain conformational differencies in actin molecules in the preparations that leads to the better digestion of actin preparations from myocardium affected by DCMP. At the same time the absence of differencies in the spectrum of proteolytic fragments suggests the absence of changes in the primary structure of proteins.

In the present paper we had shown the presence of aAbs directed against two tissue-specific myocardial proteins — actin and myosin.

In the recent investigations the aAbs directed against ANT and BCKD from myocyte mitochondria as well as β-adrenoreceptor [4—8] was described. The aAbs directed against myosin were detected only at experimentally induced myocarditis in mice [19]. The investigations of autoantibodygenesis against actin and myosin put forward several important questions. First, myosin and actin represent the intracellular antigens. The origin of aAbs against them as well as aAbs against any other intracellular is unclear [20].

It seems possible that aAbs directed against intracellular antigens may be induced by viral infection, for example, Coxaki CB3, that may induce myocarditis in mice [1, 21].

The overlapping peptides from myosin were synthesied and with a help of them the autoimmune epitope of the heavy chain of α-isoform of myosin had been localized. It was also shown that myocarditogenic forms do not possess crossreactivity with myosin [1, 9]. Kanningham had shown the immunological crossreactivity among several α-spiralic proteins including myosin, tropomyosin, vimentin, M-protein from streptococcus and laminin, the surface protein from cardiomyocytes with a help of monoclonal antibodies [1]. The peptides from *M*-protein had shown the high structural homology with myosin and laminin.

It's likely that in humans the progression of disease is more complicated then in mice. Kandolf had shown during the last stages of myocarditis and DCMP the limited replication of viral RNA, and Weiss had not detected CB3 by PCR method [1]. So we can consider autoimmune mechanisms sufficient in the origin of DCMP pathogenesis.

One of the possible explanations of the origin of autoimmune diseases is that viral infection [20, 21] leads to the secretion of antigen which are identified by «professional» antigen-presenting dendrite cells in cardial matrix. Moreover, in humans the degree of cellular inflammation and rigidity of myocyte destruction doesn't correlate exactly with cardial disfunction that is in order with suggestion of aAbs directed against myocyte antigen participation in the pathogenesis of myocarditis and DCMP.

It seems reasonable to focuse our investigations on the heavy myosin chain as the most immunogenic part of the molecule. It's a problem of special interest because in mice the immunization by myosin heavy chain induced the myocarditis that was similar to that caused by Coxaki virus CB3. At the same time it's well known that in many cases myocarditis leads to the progression of severe DCMP forms and aAbs spectra are very similar at these pathologies. Moreover, the investigation of this myosin epitope is sufficient not only from the point of view of search of target antigen while cardiomyopathy development but also helps in understanding of the functional role of aAbs directed against tissue-specific myocardial antigens at DCMP. Even in the case of described auto-

antigens at DCMP (as ANT or β-adrenoreceptor) the function of aAbs in the origin and pathogenesis of disease is unclear yet.

It's obvious that the study of structure and properties of autoantigens and their possible modifications must be conducted simultaneously. This may help us in the understanding of the other aspect of autoimmune disease development: what leads to the immunogeneicity of autoantigen leading to the absence of tolerance to «own» antigens? We consider important the further investigation of antigens from normal and affected by DCMP myocardium, and the same antigens from skeletal and smooth muscle by conformational methods such as spectrofluorimetry, circular dichroism, electronic paramagnetic resonance and so on.

The further investigation of autoantibodygenesis against the components of contractile apparatus of myocytes may help to obtain the new information about actin and myosin functioning because aAbs are often directed against another epitopes of the same antigen that are evolutionary conserved and possess functional importance [22]. The data of our investigations may help not only to understand the mechanisms of DCMP development but other heart diseases as well.

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ДОСЛІДЖЕННЯ АУТОАНТИТІЛ ДО ТКАНИНОСПЕЦИФІЧНИХ АНТИГЕНІВ МІОКАРДА ПРИ ДИЛЯТАЦІЙНІЙ КАРДІОМІОПАТІЇ

Резюме

У роботі досліджено аутоантитіла проти тканиноспецифічних антигенів міокарда людини — актина і міозина. Методом твердофазного імуноферментного аналізу (ELISA) показано, що імунореактивність сироваток крові пацієнтів з дилятаційною кардіоміопатією (ДКМП) проти досліджених антигенів суттєво вища, ніж у здорових донорів. При порівнянні імунореактивності однакових сироваток проти антигенів, виділених із нормального і ураженого ДКМП міокарда, виявлено наступну закономірність: імунна відповідь на міозин із нормального міокарда була слабшою, ніж на той же білок із міокарда, ураженого ДКМП. Для актина спостерігалася зворотна залежність. Аналіз актина із нормального і ураженого ДКМП міокарда за допомогою методу обмеженого протеолізу дозволяє вважати, що з розвитком ДКМП цей білок піддається субмолекулярним (скоріш за все, конформаційним) модифікаціям, які призводять до змін його імунореактивності. Отримані результати обговорюються з позиції функціонування скорочувального апарату кардіоміоцитів.

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