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DYNAMICS OF THE NK CELL CYTOLYTIC ACTIVITY IN ACUTE MYOCARDIAL INFARCTION: THE ROLE OF CIRCULATING IMMUNE COMPLEXES

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NK cells play an important role in the pathogenesis of atherosclerosis, using their cytotoxic mechanisms that promote the initiation, progression, and rupture of atherosclerotic plaques that leads to acute myocardial infarction. However the data about their cytolytic activity in post-infarction period remain controversial.

The aim of this work was to study the dynamics of changes in the NK cell cytolytic activity within three weeks after acute myocardial infarction in connection with the level of circulating immune complexes in the peripheral blood.

Material and methods. 16 healthy donors and 35 patients after the development of acute myocardial infarction (AMI) on the 1st, 7th and 21st days was studied for the NK cell cytolytic activity of the mononuclear fraction of peripheral blood, evaluated by the cytolysis of erythroleukemia K-562 cells *in vitro* and levels of serum circulating immune complexes (CIC) using the method of precipitation with polyethylene glycol-6000.

Results and discussion. In the most patients, AMI led to functional anergy of NK cells, but in the smaller part of patients, the increase in their cytotoxicity was observed. The post-infarction period was characterized by an increased content of CIC, the level of which positively correlated with the development of functional anergy of NK cells.

Possible mechanisms for the development of both functional anergy and an increase in NK cell activity in the post-infarction period are discussed.

Keywords: acute myocardial infarction, NK cells, circulating immune complexes.

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Т Ұ Ж Ы Р Ы М

ЖЕДЕЛ МИОКАРД ИНФАРКТІСІ КЕЗІНДЕГІ NK ЖАСУШАЛАРЫНЫҢ ЦИТОЛИТИКАЛЫҚ БЕЛСЕНДІЛІГІНІҢ ӨЗГЕРУ ДИНАМИКАСЫ: АЙНАЛЫМДАҒЫ ИММУНДЫҚ КЕШЕНДЕРДІҢ РӨЛІ

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NK жасушалары өткір миокард инфарктісіне (ӨИМ) әкелетін атеросклеротикалық бляшкалардың басталуына, өршуіне және жарылуына қатысатын өзінің цитотоксикалық механизмдерді қолдана отырып, cells атеросклерозының патогенезінде маңызды рөл атқарады. Алайда олардың инфарктік кезеңнен кейінгі цитоликалық белсенділігі туралы деректер өз ара қайшы ретінде қалып отыр.

Бұл жұмыстың **мақсаты** перифериялық қандағы айналымдағы иммундық кешендердің (АИК) деңгейіне байланысты миокард инфарктісінен кейінгі алғашқы үш аптада NK жасушаларының цитолитикалық белсенділігінің өзгеру динамикасын зерттеу болды.

Материал және әдістері. 1-ші, 7-ші және 21-ші тәулікте ӨИМ дамығаннан кейін 35 науқаста және 16 сау донорда in vitro жағдайында К-562 эритролейкемия жасушаларының цитолиз реакциясында бағаланатын перифериялық қанның мононуклеарлы фракциясының NK-жасушаларының цитолитикалық белсенділігін және полиэтиленгликоль-6000 преципитация әдісінің көмегімен қан сарысуындағы АИК құрамы зерттелді.

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Нәтижелері және талқылауы. Көптеген науқастарда ӨИМ NK жасушаларының функционалды анергиясына, ал кішілерінде олардың цитотоксикалық өсуіне әкелді. Инфаркттан кейінгі кезең АИК құрамының жоғарылауымен сипатталды, олардың деңгейі NK жасушаларының функционалды анергиясының дамуымен оң байланысты болды. Функционалды анергияны дамытудың және инфаркттан кейінгі кезеңде NK жасушалық белсенділігінің артуының мүмкін механизмдері талқыланады.

Негізгі сөздер: жедел миокард инфарктісі, NK-жасушалар, айналымдағы иммундық кешендер.

Р Е З Ю М Е

ДИНАМИКА ИЗМЕНЕНИЯ ЦИТОЛИТИЧЕСКОЙ АКТИВНОСТИ NK-КЛЕТОК ПРИ ОСТРОМ ИНФАРКТЕ МИОКАРДА: РОЛЬ ЦИРКУЛИРУЮЩИХ ИММУННЫХ КОМПЛЕКСОВ

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NK-клетки играют важную роль в патогенезе атеросклероза cells, используя свои цитотоксические механизмы, которые участвуют в инициации, прогрессии и разрыва атеросклеротических бляшек, приводящего к острому инфаркту миокарда (ОИМ). Однако данные об их цитолитической активности в постинфарктный период остаются противоречивыми.

Целью этой работы явилось изучение динамики изменения цитолитической активности NK-клеток в первые три недели после инфаркта миокарда в связи уровнем циркулирующих иммунных комплексов (ЦИК) в периферической крови.

Материал и методы. У 35 больных после развития ОИМ на 1-е, 7-е и 21-е сутки и 16 здоровых доноров изучали цитолитическую активность NK-клеток мононуклеарной фракции периферической крови, оцениваемую в реакции цитолиза клеток эритролейкемии К-562 в условиях *in vitro*, и содержание в сыворотке крови ЦИК с помощью метода преципитации полиэтиленгликолем-6000.

Результаты и обсуждение. ОИМ у большей части больных приводил к функциональной анергии NK-клеток, а у меньшей – к нарастанию их цитотоксичности. Постинфарктный период характеризовался повышенным содержанием ЦИК, уровень которых положительно коррелировал с развитием функциональной анергии NK-клеток. Обсуждаются возможные механизмы развития как функциональной анергии, так и возрастания NK-клеточной активности в постинфарктный период.

Ключевые слова: острый инфаркт миокарда, NK-клетки, циркулирующие иммунные комплексы.

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Cardiovascular failure, which is the most important cause of death worldwide, is mainly associated with atherosclerosis, a multifactorial and very complex disease, accompanied by the participation of innate and adaptive immune cells in the progression of myocardial damage [1]. Acute myocardial infarction (AMI), which occurs due to acute coronary artery ischemia resulting from rupture of an atherosclerotic plaque followed by thrombosis, leads to a life threat and the development of coronary heart disease [2, 3]. Damaged cardiomyocytes, as a result of AMI, release a combination of alarmins, chemokines, and cytokines, which, in turn, recruit populations of pro-inflammatory cells from the systemic circulation, in which neutrophils and monocytes predominate, forming sterile myocardial inflammation. Natural killer (NK) cells are the minor part of the pool of innate immunity cells, participating in myocardial inflammation [4]. Subsequently, as the autoimmune response to autoantigens from the necrotic myocardium develops, cells of the adaptive immune system (T and B lymphocytes) join them [5]. As a result, the formation of anticardial antibodies occurs, which leads to the formation of circulating immune complexes (CICs) [6], the amount of which was found to correlate with the mass of necrotic tissue [7]. The population composition of T-lymphocytes in cardiomyopathy, including AMI [8], is intensively studied and actively debated. The activity and role of NK cells in the pathogenesis of coronary heart disease remains obscure and controversial.

The aim of this work was to study the dynamics of changes in the cytolytic activity of NK cells within three weeks after AMI in connection with the level of CICs in the peripheral blood.

MATERIAL AND METHODS

We examined 35 patients who had myocardial infarction (mean age 48.3 ± 5.5 years) and 16 healthy people (control group), comparable in age and sex. All patients and healthy people gave their written informed consent before enrollment according to the Declaration of Helsinki. The diagnosis of AMI was made on the basis of clinical, electrocardiographic and biochemical parameters. The study did not include the patients, who had, at the time of admission to the hospital, complications such as true cardiogenic shock, pulmonary edema, severe disturbances of conduction and heartbeat (AV blockade of 3rd grade, paroxysmal tachycardia). Primary focal AMI was diagnosed in 30 patients, and in 5 patients, myocardial infarction was repeated. According to localization, anterior myocardial infarction was established in 25 patients, posterior diaphragmatic - in 10. There were complications in the form of disturbance of conduction and heartbeat (17 patients), heart failure (15 patients), left ventricular myocardial aneurysm (10 patients), acute left ventricular failure (10 patients), recurrence of myocardial infarction (5 patients).

Patients received standard therapy, including nitrates, angiotensin-converting enzyme inhibitors, anticoagulants, antiplatelet agents, non-narcotic and narcotic analgesics.

All patients underwent general clinical and biochemical examination upon admission to the hospital and in the dynamics of observation, including a general blood analysis, the activity of aspartate aminotransferase, alanine aminotransferase and the content of myoglobin in the blood, as well as electrocardiography, echocardiography and coagulogram.

The cytolytic activity of NK cells of the peripheral blood mononuclear fraction was determined on the 1st, 7th and 21st day of the disease. The mononuclear fraction of cells was isolated from whole venous heparinized blood by isopycnic centrifugation on a density gradient of Histopack 1077 (Sigma-Aldrich, Germany) at 3000 g for 20 min at 4°C. The cells of the interphase ring were washed with a 20-fold volume of RPMI-1640 medium (Sigma-Aldrich, Germany) and centrifuged at 300 g for 15 min at 20°C. After this, the monocytes were depleted by two-hour adhesion on glass Petri dishes. NK cell activity was determined using the Cellular DNA Fragmentation ELISA kit (Sigma-Aldrich, Germany) according to the manufacturer's instruction, by culturing mononuclear cells at 37 °C and 5% CO2 in round-bottom plates in RPMI-1640 medium supplied with 10% of fetal bovine serum (Sigma-Aldrich, Germany), together with bromodioxiuridine-labeled erythroleukemia K-562 cells in a ratio of 20:1. After 4 hours of cultivation the content of labeled DNA was determined in the cell cultures supernatant, using monoclonal antibodies to bromodioxiuridine by enzyme-linked immunosorbent assay. The cytotoxicity index (CI) of NK cells was calculated by the formula: (E-C)/Cx100%, where E is the experiment, C is the control (target cells without adding mononuclear cells).

CICs were determined in blood serum according to the method of M.Digeon et al. (1977), using for their precipitation polyethylene glycol 6000 (Merck, USA) followed by nephelometry of the solution by a spectrophotometer.

For statistical processing, the Microsoft Excel application program was used. The arithmetic mean (M), standard deviation (Sd), and the correlation coefficient (r) were calculated. The statistical significance of differences in the means was evaluated by Student t-test at a significance level of $p<0.05$.

RESULTS

A study of the NK cell cytotoxicity of healthy donors showed a low CI level (5.8±3.7%), however, non-zero. In the group of patients with AMI on the 1st day of observation in 15 of 35 cases (42.9%), there was a complete absence of NK-cell cytotoxicity (CI = 0%). In the remaining 20 patients (57.1%), the average CI level reached 12.1±2.8%. On the 7th day there was an increase in the number of patients with "zero" CI (22 of 35 - 61.5%). Moreover, in the remaining 13 patients (38.5%), the level of NK-cell cytotoxicity increased significantly (CI = 18.4± .9%). On the 21st day the absence of NK cell cytotoxicity was detected in 28 patients (80%). In the remaining 7 patients the average CI was 24.7±4.1%. The results of the analysis of blood NK cells of patients with "non-zero" cytolytic activity are presented in Figure 1.

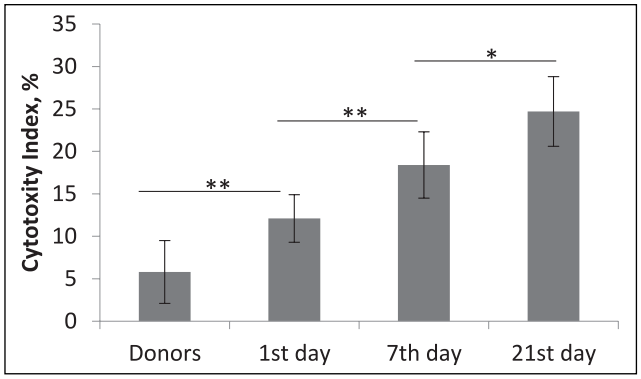

Figure 1 - Dynamics of the cytolytic activity of peripheral blood NK cells in patients with "non-zero" CI after acute myocardial infarction. Designations: * P<0.01, ** P<0.001

Analysis of the occurrence of "zero" and "non-zero" NK cell cytolytic activity among patients in the dynamics of observations showed an increase in the proportion of the patients with lack of NK cell ability to lyse target cells from 42.9% (1st day) to 80% (21st day) (Fig. 2).

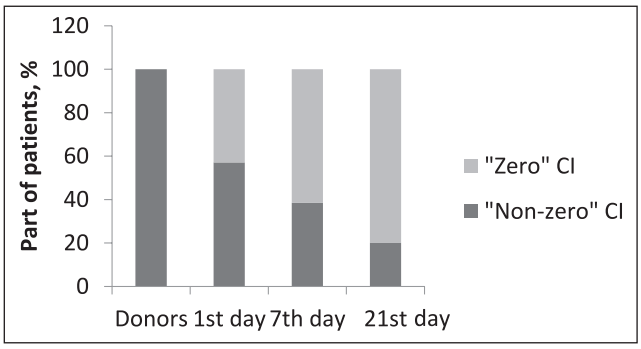

Figure 2 - The occurrence of "zero" and "non-zero" cytolytic activity of peripheral blood NK cells among patients with AMI in the dynamics of observations

The analysis of CICs is presented in Figure 3. Already on the 1st day in the examined patients, the level of CICs significantly ($p<0.05$) exceeded its value in the control group

(1.60±0.15 g/l against 1.13±0.02 g/l). CIC level was increased on the 7th day after AMI (2.15±0.18 g/l), remaining at a high level on the 21st day of observation (2.0±0.15 g/l).

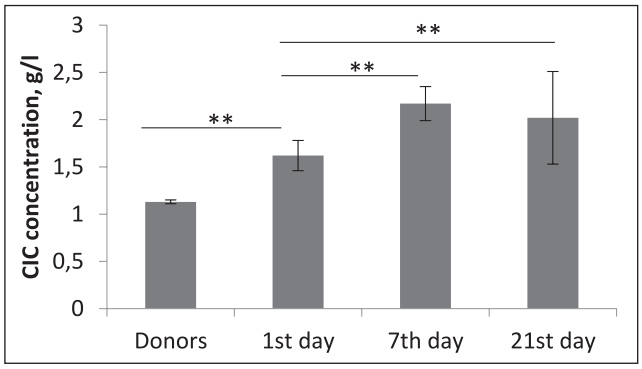

Figure 3 - Dynamics of changes in the content of circulating immune complexes in the peripheral blood serum of patients with acute myocardial infarction. Designations: ** P<0.001

DISCUSSION

NK cells are a subpopulation of innate lymphoid cells with pronounced cytolytic activity integrated into the effector link of innate immunity during the antiviral, antitumor, antimicrobial, and antiparasitic immune responses [9]. It has been suggested that NK cells play an important role in the pathology of atherosclerosis using their cytotoxic mechanisms that promote the initiation, progression, and rupture of atherosclerotic plaques [10], which indicates the integration of NK cells associated with plaques into the pathogenesis of AMI, although the exact mechanism of this process remains unknown. A number of researchers, however, note a decrease in the cytolytic activity of circulating NK cells in atherosclerosis, as well as in AMI [11, 12]. In our study, divergence of the cytotoxicity index of NK cells was observed in a cohort of examined patients. In the majority of patients, a decrease in CI occurred up to the full functional anergy of NK cells ("zero" CI). In the minor part of patients, CI, on the contrary, increased. Obviously, the mechanisms of these opposite processes were significantly different. A comparative analysis of the dynamics of changes in the proportion of the studied patients with functional anergy of NK cells and the level of CICs revealed a high positive correlation (r = 0.70) between these indicators, which suggests the involvement of CICs into suppression of the cytolytic activity of NK cells. The direct inhibitory cytolytic activity of human NK cells by the action of immune complexes *in vitro* was shown in earlier study [13]. Obviously the binding of the immune complex to NK cells was due to the interaction of the low affinity FcγRIII receptor (CD16) with the IgG Fc fragment. Previously, it was shown that binding of CD16 to antibodies led to functional anergy of NK cells [14] that supports the assumption of the negative effect of CICs on the NK cell cytolytic activity.

As for the increase in the cytolytic activity of NK cells in the minor part of patients, it can be assumed that it could be caused by IL-2 stimulation, a high level of which was observed in patients in the post-infarction period [4, 15]. As it is known, IL-2 is a natural activator of NK cells [9]. Since NK cells are an essential part of antiviral protection, patients with high levels of NK cell activity are obviously less likely to undergo a viral infection in the post-infarction period.

Based on these considerations, it is possible to assume the feasibility of including in the therapeutic regimen for patients in the post-infarction period the preparations based on the recombinant IL-2 (e.g., Roncoleukin®) for stimulating antiviral immunity. In addition, IL-2 may be useful for the activation of T-regulatory (Treg) cells responsible for the natural immune tolerance to autoantigens, which, in turn, will lead to a decrease in the level of CICs with autoantibodies to myocardial antigens. It was shown that low doses of IL-2 in vivo led to an increase in the number of circulating CD4+CD25+-FOXP3+ Treg cells [16]. In 2017, clinical trials of low doses of Aldosleukin® (recombinant IL-2) for the treatment of stable coronary heart disease and acute coronary syndromes with a detailed analysis of the state of cellular and humoral immunity, including the level of circulating NK cells, started [17].

It seems important to conduct further studies of the quantitative and functional parameters of NK cells in combination with an assessment of the cytokine level that regulate their activity in patients with AMI depending on the clinical picture and post-infarction complications in the form of infectious diseases, including long-term follow-ups.

CONCLUSIONS

1. Acute myocardial infarction leads to divergence of the cytolytic activity of NK cells circulating in the blood: in most patients, the functional anergy of NK cells gradually develops, and in the smaller, their cytotoxicity increases.

2. The post-infarction period is characterized by an increased content of circulating immune complexes in the blood, the level of which positively correlates with the development of functional anergy of NK cells.

3. It is assumed that the development of functional anergy of NK cells in patients with a post-infarction period is associated with ligation of IgG immune complexes with low affinity FcγRIII (CD16) receptors for NK cells, and the increase of their cytolytic activity is associated with the action of IL-2, the level of which, by literature data, increases in patients after acute myocardial infarction.

Research transparency

Research did not have a sponsorship.

Declaration about financial and other relations

The release script was approved by all authors. The authors did not get the honorary for the article.

Conflict of interest

The authors declare no conflict of interest.

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