# Cryoglobulin Composition in Patients with Myocardial Infarction

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The formation of an ischemic focus in the myocardium is paralleled by autoimmune processes involving immunoglobulins with abnormal thermal solubility, cryoglobulins. Serum levels of cryoglobulins and their complexes were measured in 25 patients with *Q*-forming myocardial infarction on days 1, 2, 3, 7, and 14 of the disease. Cryoprotein levels were measured by spectrophotometry, concentrations of IgM, IgA, IgG, and fibronectin by ELISA. Cryocomplexes in patients with myocardial infarction consist of polyclonal immunoglobulins of the main classes IgG and IgM, less often IgA, while cryoglobulinemia in this patient population is referred to type III according to the commonly used classification by Brouet. Fibronectin was detected in the cryocomplexes throughout the entire period of observation, its dynamics being opposite to that of the cryocomplexes level.

**Key Words:** *myocardial infarction; cryoglobulins; cryocomplexes; fibronectin*

The formation of an ischemic focus in the myocardium is paralleled by autoimmune processes with participation of immunoglobulins with abnormal thermal solubility, cryoglobulins (CG), forming immune complexes with cryoproperties (ICC). Despite intensive recent studies of the pathogenetic role of CG and ICC in ischemia [2,4], many problems of the functional relationship between cryoproteins and their complexes, qualitative and quantitative composition thereof, mechanisms of their formation and elimination, contribution to autoimmune reactions, specifically to acute myocardial ischemia, remain unclear.

We evaluated qualitative and quantitative composition of CG and their complexes in patients with myocardial infarction (MI) and studied the dynamics of these parameters during treatment.

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## MATERIALS AND METHODS

A total of 25 patients (12 women and 13 men) aged 30-54 years with acute *Q*-forming MI, hospitalized within the first 24 h of the disease in cardioresuscitation block of N. I. Pirogov Hospital No. 1, were examined. The reference group consisted of 18 donors of similar age and gender. Cryoglobulins were measured by A. E. Kalavidoris' method. The blood was collected from the cubital vein by gravity into warm (37°C) sterile tubes. The clot formed in a thermostat (37°C). After centrifugation, the serum was placed into a refrigerator and kept at 4°C for 7 days. After repeated centrifugation and separation from the supernatant, the precipitate was washed 3 times in phosphate buffered saline and dissolved in the same buffer to the initial volume of the serum.

Quantitative evaluation of cryoproteins was carried out on a Carry-50 spectrophotometer at $\lambda$=280 nm with extinction coefficient of 1.4 by the difference of optical densities: $\Delta OD=OD_4-OD_{37}$, where $OD_4$ and $OD_{37}$ were optical densities of CG in the buffer at 4 and 37°C. Calculation by the difference in optical den-

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sities rules out possible impact of immune complexes without cryoactivities.

Qualitative composition of ICC was evaluated by vertical PAAG electrophoresis with sodium dodecylsulfate under reducing and nonreducing conditions with subsequent staining of the gel with Coomassi Bright Blue R-250 on a Mini-Protean 3 vertical electrophoresis device (BioRad).

The CG composition was evaluated over the course of observation. The concentrations of IgM, IgA, IgG, and fibronectin were measured by ELISA using commercial test systems and reagents (Biosource, Vector-Best, Bender MedSystems).

The reaction results were evaluated at λ=492 nm on a Pikon EIA reader. The concentrations of the substances were evaluated by calibration curves.

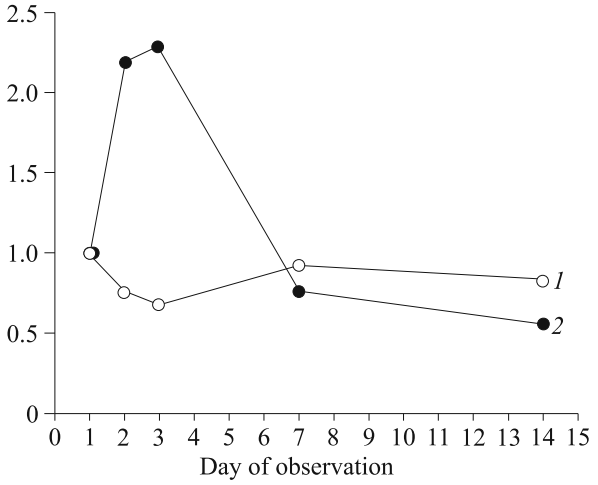


**Fig. 1.** Changes in ICC (*1*) and fibronectin (*2*) concentrations in ICC in MI.

## RESULTS

Cryoglobulins in high concentrations were found in 100% examined patients from day 1 to day 14 (normal values 60-80 μg/ml). The maximum levels were recorded on day 1 and minimum values on day 3 of the disease (Fig. 1).

The dynamics of total ICC and their components was analyzed by comparing all the studied parameters to their initial values in each patient on day 1 of observation. Initially high level of cryoproteins on day 1 of MI reflects preinfarction sensitization at the expense of chronic inflammation of the vascular endothelium in atherosclerotic involvement of the arteries. This inflammation is paralleled by progressive formation of antibodies to atherosclerotic plaque components, expression of various polyvalent peptides (heat shock proteins, *etc.*), cytokine imbalance, and extracellular matrix restructuring (with participation of matrix metalloproteinases, *etc.*) [3]. All these processes provoke prolongation of inflammation and lead to active formation of immune complexes, including those with cryoproperties [7].

A significant relationship ($p<0.02$) between the dynamics of cryoglobulinemia and CG level on admission to hospital attests to a certain phase pattern of the dynamics of cryoglobulinemia even before the development of acute myocardial ischemia.

Cryoglobulin levels in circulating blood decreased on day 2 and further decreased on day 3 of MI development and after the beginning of therapy. This can be explained by therapy and stimulation of anti-inflammatory mechanisms of the immune system, as a result of which autoantigens, additionally released into circulating blood, formed autoimmune cryocomplexes, which were actively eliminated from the body primarily due to stimulation of the complement system and phagocytic system [1,7,12].

By day 7 of MI, the blood level of CG increased virtually to values recorded on day 1 of the disease. The second wave of cryoglobulinemia presumably reflected autoimmune response to the formation of the ischemic focus in the myocardium [8,9]. Elimination of CG was evaluated by reduction of their level by day 14.

Analysis of qualitative composition of ICC in MI patients showed that IgM, IgG, and fibronectin were present throughout the entire period of observation in 100% patients, while IgA was found in ICC on day 1 in only 77% and by day 14 in 89% patients (Fig. 2). Hence, the components of ICC in MI patients are polyclonal Ig of the main classes IgG, IgM, and less often IgA. Cryoglobulinemia in patients with this condition is classified as type III according to the commonly used Brouet classification.

Quantitative composition of ICC (the main CG IgM, IgG, IgA CG and fibronectin) was evaluated on day 1 of MI and over the course of therapy (Figs. 2 and 3, respectively). On day 1, IgG and IgA predominated, IgM content being lower (Fig. 2). The content of IgG was 2- and 3-fold higher than the levels of IgA and IgM, respectively, which correlated with serum concentrations of IgG, IgA, and IgM in donors. The predominance of IgG in comparison with other Ig in cryocomplexes of MI patients indicates lasting pre-infarction sensitization associated with immune response to atherosclerotic involvement of vessels. In addition, IgG antibodies, which predominated (particularly in secondary immune response), had certain advantages over IgM antibodies due to their affinity for the antigen and the effector and regulatory functions.

By day 3 of the disease, the immunoglobulin constituent of ICC changed. The IgA and IgM predomi-

nated, while IgG level was lower than those of IgA and IgM. Hence, the cryocomplex was transformed, which inevitably led to modification of its functional characteristics and activity. By day 14, the levels of all the studied CG tended to return to the initial level (Fig. 3). The dynamics of the main cryoimmunoglobulins in the cryocomplex differed from the dynamics of total CG level.

We showed that fibronectin is present in ICC of all patients during all periods of observation. This indicates its exceptionally high functional significance in the manifestation of pathogenetic properties of ICC. Fibronectin, the main blood opsonine, is directly involved in absorption and elimination of pathogenic cryocomplexes, damaged collagen matrix degradation products, fibrinogen-fibrin degradation products, and in other processes [10].

The dynamics of fibronectin concentrations in ICC of MI patients during the entire period of observation is presented in Figure 1. The curve is exponential and is opposite to the dynamics of total CG level in the blood of MI patients. The maximum fibronectin level in ICC was recorded on day 3 (2.2 times higher than on day 1). A drop of this parameter were observed on days 7 and 14 (by 2.8 and 1.3 times, respectively, in comparison with the initial level of 1.8 mg/ml).

Presumably, fibronectin in the cryocomplex is modified. The fibronectin extra domain A is an endogenous ligand for Toll-like receptors (TLR) [5,13]. This is confirmed by our findings and published data indicating that fibronectin is always present in ICC in MI; the molecular weight of fibronectin in the complex differs significantly from the molecular weight of native fibronectin [6]. The TLR stimulation modifies the expression of genes essential for realization of congenital immune response and for triggering the acquired immunity reactions, as well as for regulation of these processes. Hence, ICC can be indirectly involved in stimulation of the immune system cells through TLR, thus leading to the production of various effector molecules (cytokines and other mediators of the immune response, inflammation, and regeneration) and the development and realization of congenital and adaptive immune response reactions [5,11,13].

Our findings and published data indicate that IgM, IgG, and IgA circulate in high concentrations in the blood of patients during the acute period of MI. These Ig form immune complexes with cryoproperties and lead to the development of mainly type III cryoglobulinemia according to Brouet's classification. These complexes stimulate the complement system binding to its constituents C1q and C3 [6]. Stimulation of the complement system, in turn, promotes attraction of neutrophils and monocytes to the ischemic focus [8,9]. It is known that C1q and C3

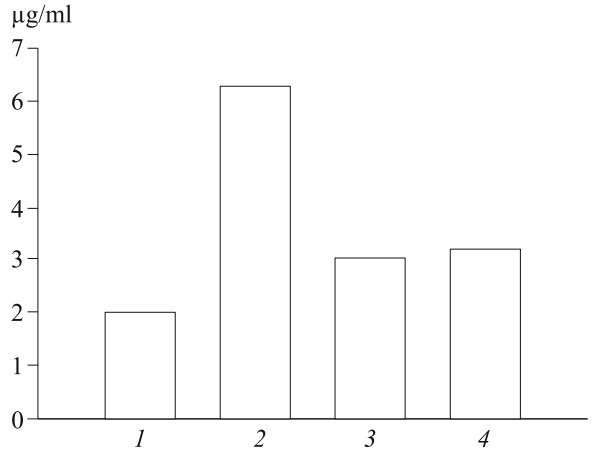


**Fig. 2.** Content of IgM (*1*), IgG (*2*), IgA (*3*), and fibronectin (*4*) in ICC on day 1 of MI.

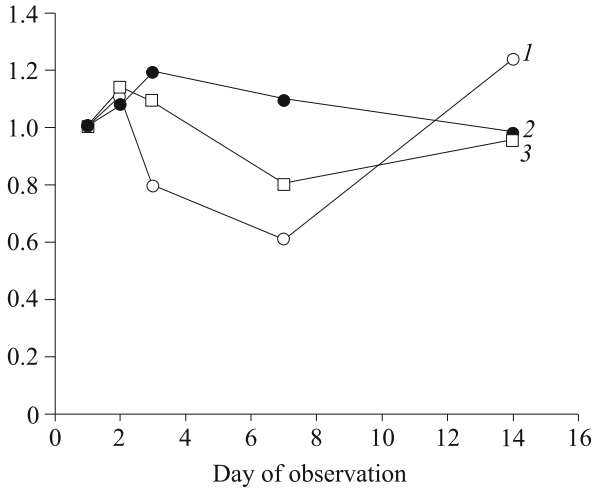


**Fig. 3.** Changes in the levels of IgG (*1*), IgM (*2*), and IgA (*3*) in ICC in MI in comparison with the levels on day 1, taken for 1.

containing immune complexes interact with phagocytic cells and are eliminated (through these cells) from circulating blood [1]. Monocytes migrating to the focus are differentiated into macrophages and not only remove dead cells (cardiomyocytes and neutrophils), but are also involved in tissue regeneration by producing growth factors and cytokines stimulating proliferation of fibroblasts and endothelial cells [8,9]. High level of fibronectin in cryocomplexes promotes stimulation of phagocytosis of damaged and pathological matrix proteins and apoptotic cells in the ischemic zone, which leads to a drop of the cryocomplexes level in circulation, especially during the first days of the disease (Fig. 1).

Hence, MI, in addition to numerous disorders in hemostasis, blood rheology, kallikrein-kinin and other neurohumoral systems, and the complement system, is associated with the appearance of ICC in high concentrations in the blood of MI patients. Qualitative and

quantitative composition of ICC changes significantly during therapy. High level of CG in the blood is a nonspecific marker of inflammatory processes and autoimmune aggression in ischemia [4]. Fibronectin in ICC plays an important role in opsonization and clearance of cryoproteins. Our results indicate that the function of CG and fibronectin is different at different stages of MI development.

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