IDENTIFICATION OF ANTIBODIES REACTING WITH HEART MUSCLE FIBER AND INTERSTITIAL CONNECTIVE TISSUE ANTIGENS WITH DIFFERENT CLASSES OF IMMUNOGLOBULINS

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Investigations by various workers have shown that antibodies reacting with antigens of different structures of the muscle fiber and blood vessel walls in the human myocardium can be detected by the indirect immunofluorescence (IF) method in patients with rheumatic diseases and myocarditis [3, 4, 7, 10, 12]. When these sera were tested by the same method on sections of the myocardium of animals, antibodies reacting with interstitial connective tissue cells of the heart also could be found [3, 7, 12].

The identity of antibodies reacting with the various structures of the myocardium with individual classes of immunoglobulins (Ig) has not been adequately studied, although there is evidence of the discovery of antibodies against components of the nuclei which belong to the IgG, IgA, and IgM classes [5, 14].

The presence of deposits of Ig belonging to different classes in pathologically changed areas of the muscle fiber and interstitial connective tissue of the myocardium in rheumatic fever also has been described, as well as in the skin, microcirculation, glomerular apparatus of the kidneys, and soft tissues of the joints in certain rheumatic diseases [1, 9, 11, 13].

Investigations have shown that in autoimmune processes and, in particular, in some rheumatic diseases there is an increase in the serum concentration of individual classes of Ig [6, 9].

In the accessible literature no data could be found on the frequency of discovery of antibodies belonging to the IgG, IgA, and IgM classes against structures of the myocardium, depending on the titer of Ig of the corresponding classes in patients' sera.

The object of this investigation was to study the frequency of discovery and the level of the titer of antibodies of the IgG, IgA, and IgM classes depending on their specificity toward antigens of different myocardial structures, the nosological form, the duration of development of the pathological process, and also the level of the corresponding Ig classes in the subjects' sera.

EXPERIMENTAL METHOD

Sera of 190 patients with rheumatic diseases – 81 with rheumatic fever, 48 with systemic lupus erythematosus (SLE), 30 with rheumatoid arthritis (RA), other rheumatic diseases (periarteritis nodosa, dermatomyositis, Sjogren's disease, systemic scleroderma) in 31; 26 sera obtained from patients with myocarditis aged from 14 to 50 years were tested by the indirect IF method.

Pure antibodies against human IgG, labeled with fluorescein isothiocyanate, were used in the indirect IF test.

Sera were studied on unfixed sections of the heart of a person with blood group O (I) and bovine heart, cut in a cryostat at −20°C. The technique of preparation of pure antibodies against IgG and the principles of processing of the sections of myocardium were described more fully previously [3].

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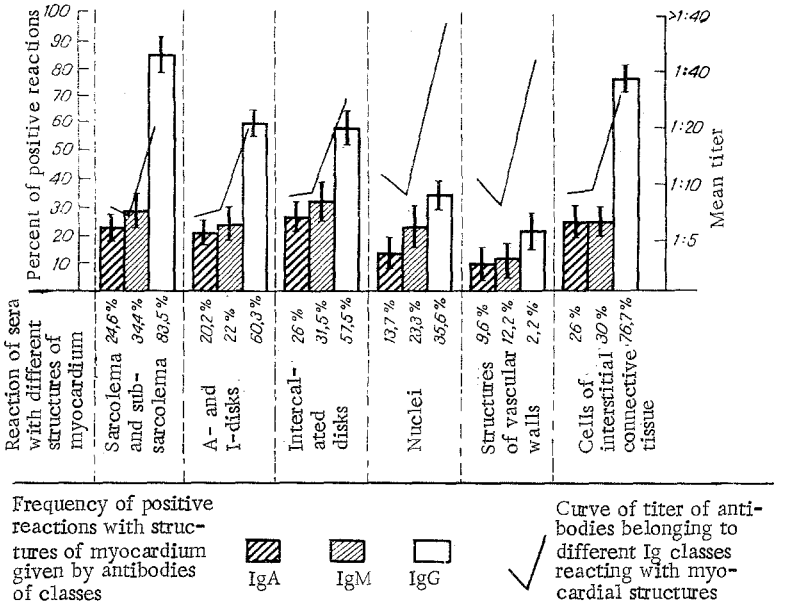

Fig. 1. Frequency of positive reactions and height of titer of antibodies of classes IgG, IgM, and IgA, reacting with individual structures of the myocardium.

Sera of patients with rheumatic fever (21), SLE (20), and RA (15), sera from patients with other rheumatic diseases (seven), and also from patients with myocarditis (10), giving a positive IF test, were also tested under the same conditions with luminescence monospecific Ig against IgG, IgA, and IgM. The method of obtaining fluorescein-labeled monospecific Ig was described previously [2].

The preparations were examined with the ML-2 luminescence microscope with × 40 objective (water immersion). The intensity of luminescence of the structures was recorded by a system of + signs (the strongest 4+).

For quantitative determination of Ig in the sera, Mancini's radial immunodiffusion method was used, with monospecific rabbit sera against human IgG, IgA, and IgM.

From four sera of patients with SLE, IgG fractions were isolated by ion-exchange chromatography on DEAE-cellulose.

EXPERIMENTAL RESULTS

Tests on the sera of the above-mentioned groups of patients by the IF using fluorescein-labeled pure antibodies against human IgG on sections of human myocardium revealed antibodies reacting with different structures of the myocardial muscle fiber: the sarcoplasm, sarcolemma, and subsarcolemma, A- and I-disks, intercalated disks, nuclei, and structures of the wall of the myocardial vessels. In bovine heart, a reaction also was observed with fibroblasts of the interstitial connective tissue.

The study of these sera with the aid of monospecific labeled Ig showed that antibodies against different structures belong to different classes of Ig.

Data showing the frequency of discovery of antibodies against individual myocardial structures depending on the Ig class to which they belong are given in Fig. 1. For most structures the frequency of detection and level of the titers of antibodies of the IgG class were significantly greater ($P < 0.01$). Antibodies against nuclei and structures of the vessel wall were the exception, for a significant excess of antibodies of the IgG class was discovered only with respect to the level of the titer. The frequency of discovery of antibodies of the IgA and IgM classes was about the same for each individual structure of the myocardium, and at the titer rarely exceeding 1:10. The titer of antibodies of the IgG class was 1:40 for most structures, and for antibodies of the IgG class reacting with components of the nuclei it reached 1:640.

In the absence of positive reactions of the test sera with individual structures of the myocardium, due to antibodies of the IgG class, no antibodies against the analogous structures belonging to the IgM and IgA classes likewise were found.

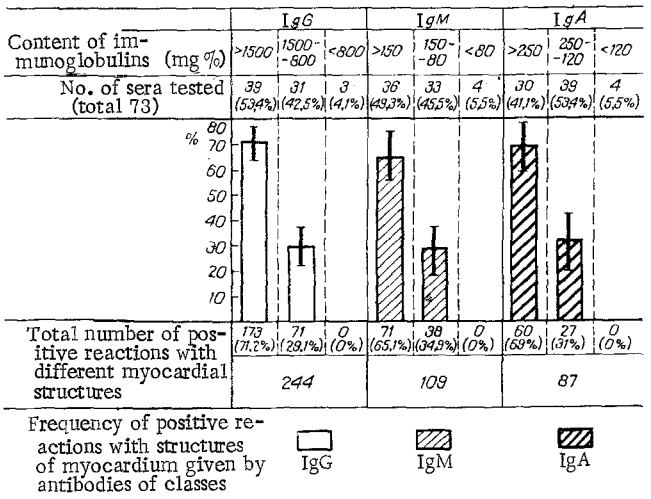

Fig. 2. Frequency of positive reactions of antibodies of IgG, IgA, and IgM classes with different structures of the myocardium depending on content of immunoglobulins of corresponding classes in sera tested.

It was also found that the presence of antibodies mainly belonging to a particular class of Ig was not characteristic of the concrete nosological form.

To rule out the possible crossed reactions of monospecific Ig, the IgG fraction was isolated from four sera from patients with SLE in which antibodies of classes IgG, IgA, and IgM were found. On subsequent testing of the IgG fraction in the IF test using monospecific labeled Ig, positive reactions were found only with anti-IgG.

On analysis of data for the individual classes of Ig to which the majority of the antibodies discovered belonged, depending on the time of development of the pathological process, the following result was obtained. Positive reactions of the test sera with myocardial structures due to antibodies of the IgG class were significantly more frequent than positive reactions of antibodies of the IgM and IgA classes, especially on the 15th-21st and 22nd-30th days after the beginning of development of the pathological process. A significant excess in the frequency of positive reactions connected with IgM antibodies was observed on the 8th-15th day of the disease. With respect to antibodies against myocardial structures belonging to the IgA class, the frequency of their discovery was higher, but not statistically significantly, at the same times as for antibodies of the IgG class.

Analysis of the frequency of positive reactions due to antibodies belonging to individual classes of Ig with structures of the myocardium, depending on the level of concentration of Ig of the corresponding classes in the sera tested gave the following results (Fig. 2).

Positive reactions were virtually completely absent during tests of sera with a low content of individual classes of Ig (IgG < 800 mg%, IgM < 80 mg%, IgA < 120 mg%). The frequency of positive reactions due to antibodies of the IgG, IgA, and IgM classes was significantly higher in the group of sera with an increased IgG content (IgG > 1500 mg%, IgA > 250 mg%, IgM > 150 mg%) compared with the group of sera with an average content of Ig (IgG 800-1500 mg%, IgA 120-250 mg%, IgM 80-150 mg%).

The results of this investigation thus confirmed the previous discovery of antibodies reacting with antigens of different structures of the muscle fiber and interstitial connective tissue of the myocardium when sera of patients with rheumatic diseases and myocarditis were tested on sections of human and bovine myocardium by the IF test [3]. The antibodies discovered, regardless of the individual myocardial structures against which they acted, included representatives of all the principal classes of Ig, although antibodies of the IgG class were significantly predominant as regards both frequency of discovery and level of titer. The absence of positive reactions of the test sera with antigens of any particular structure of the myocardium and due to antibodies of only the IgA or IgM class, will be noted.

No correlation was found between predominance of antibodies of one particular class reacting with the structures of the myocardium and the nosological form.

A definite rule was observed with respect to discovery of antibodies belonging to particular classes of Ig depending on the duration of the pathological process.

The frequency of positive reactions of antibodies with structures of the myocardium, and belonging to the IgG, IgM, and IgA classes, was found to be statistically significantly higher depending on the level of the titer of the corresponding classes of Ig in the sera tested.

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