Immunofluorescence Method for Detecting Anti-Myocardial Antibodies, and Its Use in Diagnosing Heart Disease

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Demonstration of autoimmune antibodies to myocardial tissue enables one to detect and assess cardiac disease long after abnormalities in serum enzyme activities are no longer measurable. We describe an indirect immunofluorescence procedure in which cryostat sections of rat heart (ventricle) and Evan's Blue counterstaining are used to detect anti-myocardial antibodies. Sera from patients with myocardial infarct or some other cardiac diseases reveal a distinct fluorescent staining of the sarcolemmal membrane. In contrast, sera from patients with systemic lupus erythematosus demonstrate nuclear plus diffuse staining and sera from myasthenia gravis patients show a characteristic striated staining pattern. The role of anti-myocardial antibodies in cardiac disease is discussed briefly.

Additional Keyphrases: systemic lupus erythematosus • myocardial infarction • coronary insufficiency • diagnostic aid • myasthenia gravis

Ordinarily, the clinical laboratory helps confirm suspected cardiac disease by measuring the activities of serum enzymes such as lactate dehydrogenase (EC 1.1.1.27), aspartate aminotransferase (EC 2.6.1.1), and creatinine kinase (EC 2.7.3.2) (reviewed in references 1 and 2). Activities of these enzymes increase and then decrease soon (hours to days) after tissue damage, and these changes aid in the immediate diagnosis. However, suspicion that such damage has occurred may arise long after such enzymatic measurements are useful. Consequently there is a need for a practicable diagnostic test that has a useful range of weeks, such as a test for autoantibodies to heart tissue.

The presence in serum of immunoglobulins that react with heart muscle (myocardial) antigens has been correlated with both the development and recurrence of cardiac symptoms after infarction, after cardiotomy, or after commissurotomy, as well as those associated with acute rheumatic fever (3, 4). These antibodies are detectable for as long as weeks to months after the injury, and so are diagnostically useful in a unique way.

We present a relatively simple and rapid indirect immunofluorescence procedure for detecting anti-myocardial autoantibodies and review the association between these antibodies and cardiac disease.

Materials

Rat-heart sections. Thick (4 μm) cross-sections of the ventricle wall of hearts of young adult rats were cut in a microtome cryostat at −20 °C and placed on microscope slides (Cell-Line Associates, Minotola, N.J. 08341). The slides were stored at −70 °C until used, which can be as long as six months later.

Phosphate-buffered saline. This solution consisted of 132 mmol of NaCl in 6.7 mmol/liter phosphate (725 mg of Na₂HPO₄ and 210 mg of KH₂PO₄ per liter) adjusted to pH 7.2.

Rat-heart powder. Three hearts from young adult rats were soaked in physiological saline for 1 h and then homogenized in 5 ml of acetone with a glass homogenizer. The homogenate was then centrifuged (10,000 × g, 10 min). The supernatant fluid was removed and discarded and the precipitate was washed three times with phosphate-buffered saline and then lyophilized.

Serum samples. Specimens from cardiac disease patients were obtained from a hospital, “normal” samples from clinically healthy laboratory personnel.
Evans Blue (C.I. Direct Blue 53) counterstain (Fisher Scientific, Santa Clara, Calif. 95050), 1 g/liter of distilled water.

Absorbed fluorescein conjugate. Fluorescein conjugated to goat anti-human gamma globulin (Meloy Labs, Inc., Springfield, Va. 22151) was absorbed with rat-heart powder (50 g/liter) at 4 °C for 16 to 20 h. The mixture was then centrifuged (2000 X g, 20 min) and the supernatant fluid, containing the absorbed conjugate, was removed and stored at -20 °C. For analysis, the absorbed conjugate was diluted 10-fold with phosphate-buffered saline.

Method

An indirect immunofluorescence technique based on the method of Closs and Aarli (5) was used to detect anti-myocardial antibodies in serum. Cryostat sections of the ventricular wall of hearts from young adult rats served as antigen. Patients’ sera were diluted 10-fold with phosphate-buffered saline and 50 µl of this diluted serum layered on each section. Positive and negative controls were included on the appropriate segments of each slide. The reactants were incubated at ambient temperature (about 22 °C) in a moist chamber for 30 min. Nonreactive serum components were washed away with a gentle stream of buffer from a wash bottle. Next, the slides were washed several times by first placing them in a small Coplin jar containing phosphate-buffered saline and agitating gently on an orbital shaker for 5 min. After the sections were air dried, 50 µl of absorbed fluorescein conjugate was applied to each section and the reactants were incubated for 30 min at ambient temperature in a moist chamber. The sections were again washed as described above and then placed for 5 min in a staining jar containing the Evan’s Blue solution, again rinsed with buffer, washed several times, and air dried. They were then mounted in glycerol/phosphate-buffered saline (9:1 by vol) and viewed under a Leitz fluorescent microscope with 3-mm BG 12 excitation filter and 450 secondary (barrier) filter at 400× magnification.

Results

Figure 1 (A and B) illustrates the typical fluorescent staining pattern displayed by anti-myocardial antibodies in sera from patients with known or suspected cardiac disorders. Positively reacting samples contain anti-myocardial autoantibodies that produce staining patterns that are qualitatively indistinguishable from each other. The fluorescence is predominantly localized at the sarcolemmal membrane, although there is some staining between cells. This is very similar to the staining pattern reported by Van der Geld (6). A positive reaction is unambiguous when compared with a negative control, of which Figure 2 is typical.

Distinguishing between positive and negative samples is more difficult in the absence of counterstaining. This dramatic difference can be seen by comparing Figures 1 and 2 with Figure 3 (A and B), which shows results if counterstaining was not done.

We looked for anti-myocardial antibodies in the sera of 23 patients who had positive titers for anti-DNA antibodies (which is generally diagnostic of systemic lupus erythematosus). Nine of these samples produced fluorescent patterns when reacted with the rat heart tissue. Reportedly (7), sera from a high percentage of patients with systemic lupus erythematosis contain autoantibodies identical in reactivity to those found in cardiac patients, but with our method the pattern observed is distinctly different from that for sera from cardiac-disease patients. It includes some diffuse staining of the sarcoplasm, but primarily nuclear staining of the type shown in Figure 4. None of the samples showed the distinct staining of the sarcolemmal membrane illustrated for sera from cardiac-disease patients.

Sera from myasthenia gravis patients frequently contain autoantibodies against muscle tissue. On immunofluorescence analysis, these antibodies are reported (6) to display a striated staining pattern when they are reacted with heart tissue. We confirmed this observation with sera from patients with myasthenia gravis (kindly supplied by Dr. Henry Smith, of our laboratory) by using rat-heart sections (Figure 5). The fluorescent pattern produced by sera from cardiac-disease patients clearly differs from the type of staining shown in Figure 5.

Samples that contained anti-myocardial antibodies and samples with anti-DNA antibody that reacted with heart tissue were absorbed with either rat heart or liver (50 g/liter). Myocardial-reactive antibody is completely absorbed with heart powder, while after absorption with liver the intensity of the fluorescence decreases but the results are still positive. Samples containing anti-DNA antibodies are unaffected by absorption with either tissue.

Forty-eight normal, clinically healthy donors were screened for anti-myocardial antibodies. Only one sample showed a positive anti-myocardial response; this unexplained exception was from a young woman with no known previous history of heart trauma.

Discussion

Although interpretation of this assay depends on morphological observation and is not susceptible to precise quantitation, the clinical time-span over which it can be used to detect heart disease makes it an effective complement to other types of analyses. Neither the immunoreactivity of human myocardial autoantibodies with rat heart nor the rather specific staining at the sarcolemmal membrane are new observations. However, the novel use of Evan’s Blue counterstaining decreases the nonspecific background fluorescence to such an extent that normal and abnormal staining patterns are readily distinguished. The practicability of the method for the general clinical laboratory provides wider access to this new diagnostic information.
Fig. 1. Indirect immunofluorescence staining pattern of a sample positive for anti-myocardial antibodies.
Magnifications in Figs. 1-4 is approximately 200X, in Fig. 5 approximately 800X. In Fig. 1, B is a threefold enlargement of A.

Fig. 2. Indirect immunofluorescence staining pattern of a sample negative for anti-myocardial antibodies.

Fig. 3. Indirect immunofluorescence staining pattern of positive (A) and negative (B) samples in the absence of Evan's Blue counterstaining.

Fig. 4. Indirect immunofluorescence staining pattern of serum from a patient with systemic lupus erythematosus.

Fig. 5. Indirect immunofluorescence staining pattern of serum from a patient with myasthenia gravis.
The ease with which staining patterns can be distinguished may explain why, in contrast to Das and Cassidy (7)—who did not counterstain—we find no similarity between staining patterns of sera from systemic lupus erythematosus and cardiac-disease patients, an important diagnostic distinction.

Anti-myocardial antibodies are frequently found in the serum of rheumatic fever patients, especially those with active disease (4, 8, 9). In acute stages of rheumatic fever accompanied by carditis about two-thirds of the patients give evidence of anti-myocardial autoantibodies; for those without carditis, only a fourth do so.

Damage to heart muscle or pericardial tissue consequent to surgical procedures, stab wounds, or acute myocardial infarctions results in syndromes of almost indistinguishable symptoms, variously termed post-cardiotomy, post-pericardiotomy, post-commissurotomy, and post-infarction syndromes (3, 4). About half of such patients demonstrate evidence of an autoimmune response to myocardial antigens; reported estimates range from 30 to 70% (9, 6, 10). Circulating anti-myocardial antibodies usually appear two to three weeks after the first injury, and then concentrations become so low as to be undetectable in a further three to eight weeks. On the other hand, in those cases involving a series of attacks or injuries over an extended period of time, positive responsiveness is almost immediately detectable and can persist for months or even years.

Bauer et al. (11) found that anti-myocardial antibodies may in some instances precede other clinical evidence of myocardial injury. They also reported that high titers of anti-myocardial antibodies are often observed in patients with acute myocardial infarction and less frequently in patients with coronary insufficiency without infarction. Thus, assessment of anti-myocardial autoimmunity could be helpful in differential diagnosis of these two diseases.

References