IMMUNOFLUORESCENCE STUDY OF ANTIBODIES AGAINST CONNECTIVE-TISSUE AND MUSCLE-FIBER COMPONENTS OF THE MYOCARDIUM IN RHEUMATIC FEVER PATIENTS

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A comparative immunofluorescence study was made of the sera of patients with rheumatic fever using tissue sections of the human, guinea-pig, and bovine heart. The sera reacted with the sarcolemma, sarcoplasm, disks, and intercalary disks of the muscle fiber in the sections of heart tissue from all species. However, the frequency of the reaction differed in different species. In sections of bovine heart and, to a lesser degree, guinea-pig heart a reaction with cells of the interstitial connective tissue was found.

Antibodies against the sarcolemma and sarcoplasm of the myocardial muscle fiber have been found by the immunofluorescence method in the sera of patients with rheumatic fever [2, 6, 8, 12, 13]. In rare cases reactions have been observed with smooth-muscle elements of the blood vessel wall [8, 12]. Virtually no antibodies against connective-tissue elements have been found despite the fact that deposits of bound γ-globulin have been observed in the connective-tissue structures of patients with rheumatic fever [7, 10]. The results described above were obtained by the study of sera on sections of human heart tissue. Antigens common to man and various species of animals are known to exist in several organs and tissues [5].

EXPERIMENTAL METHOD

Altogether 38 sera of adult patients with rheumatic fever were studied. Sera of patients with other diseases (15) and of donors (21) were used as the controls. The indirect immunofluorescence method with pure antibodies against human γ-globulin was used. Pure antibodies against human γ-globulin were obtained from ass serum against human globulins by the use of an immunosorbent as described by Avrameas and Ternynck [3]. Human γ-globulin, obtained by the method of Baumstark et al. [4] was converted into immunosorbent with the aid of glutaraldehyde. The immune serum was added to the immunosorbent, washed to remove nonspecific protein, and the antibodies were eluted in acid medium. The pure antibodies were conjugated with fluorescein isothiocyanate at pH 8.6. Unbound dye was removed by filtration on Sephadex G-25. The technique of preparing and labeling the pure antibodies was fully described previously [1].

The antibodies were studied on tissue sections of human, guinea-pig, and bovine heart. Pieces of guinea-pig and bovine myocardial tissue were taken from the wall of the left ventricle of the healthy animals. For testing the sera on sections of the human heart, biopsy material was used: tissue of the auricle obtained at commissurotomy, and to exclude reactions with isoantibodies, tissue from persons with blood group O only were used. The pieces of tissues were frozen in petroleum ether at $-70°C$. Sections were cut to a thickness of 4μ in a cryostat at $-20°C$. In most experiments unfixed tissue sections were used. Fixation with acetone had no effect on the results of the tests. The sera was preserved at $-20°C$ and tested in dilutions of 1:4 and 1:6. To abolish nonspecific luminescence the sera and labeled antibodies were twice adsorbed with rabbit liver powder. Sections of animal heart tissue were incubated for 45 min

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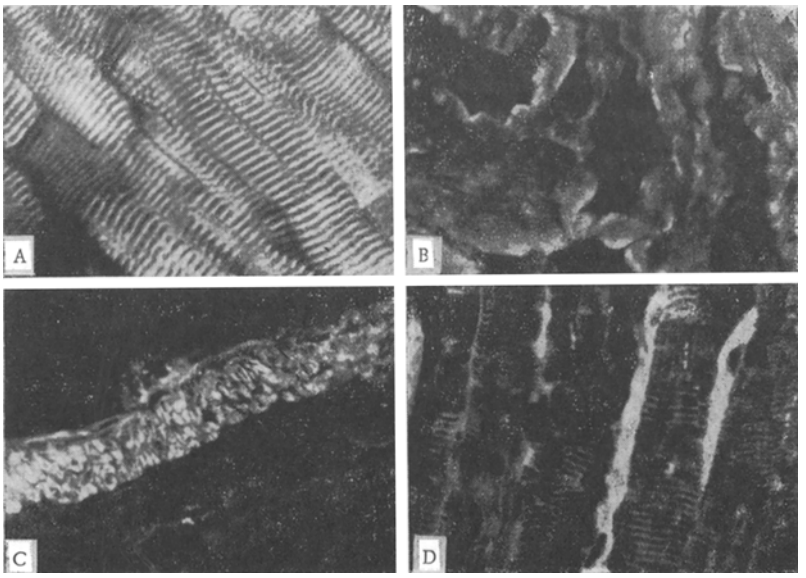

Fig. 1. Reactions of sera of rheumatic fever patients with heart tissue sections: A) luminescence of muscle fiber disks (section of bovine heart tissue); B) luminescence of sarcolemma and adjacent zones of sarcoplasm (section through guinea-pig heart tissue); C) luminescence of smooth-muscle elements of vessel wall (section through guinea-pig heart tissue); D) luminescence of interstitial connective-tissue cells and muscle fiber disks (section through bovine heart tissue). A, B, D) objective 40 ×, homal 5 ×; C) objective 40 ×, homal 3 ×.

with serum and 30–40 min with labeled antibodies. The sections were washed with buffered 0.85% NaCl solution, pH 7.0. When the action of the sera on human heart sections was tested technical difficulties arose, mainly because the labeled antibodies reacted directly with the tissue section, evidently on account of γ-globulin which had not been completely removed by washing the section for 30 min at pH 7.2–7.4. When the experiment was carried out under the usual conditions (room temperature, incubation for 45 min with serum and 30–40 min with labeled antibodies) the sera of rheumatic fever patients reacted only very weakly with the heart tissue sections. The best results were obtained when the sera were applied to unwashed sections and incubation was carried out for 1 h at room temperature and for 18 h at 4°C [9]. The sections were washed for 10 min with buffered 0.85% NaCl solution, pH 7.2–7.4, and stained for 1 h with labeled antibodies against human γ-globulin. The finished preparations were mounted in buffered glycerol, pH 7.0, and studied in the ML-2 luminescence microscope with 40 × objective (water immersion).

EXPERIMENTAL RESULTS

When sera of rheumatic fever patients were tested on human, guinea-pig, and bovine heart tissue sections reactions were observed with various elements of the myocardial muscle fiber. Besides reactions with the sarcolemma and sarcoplasm (Fig. 1B), luminescence of the disks (Fig. 1A) and intercalary disks was observed. In some cases reactions also were found with the smooth muscle of the vessel wall (Fig. 1C).

The frequency with which reactions with the various structures were detected when the sera were tested on tissues of the various species was not the same in every case. About 30% of the sera reacted with sarcolemma when tested on human heart tissue sections, but only isolated sera reacted with this structure when tested on animal tissue sections. More than half of the sera tested reacted with muscle fiber sarcoplasm, and the reaction was found in approximately the same frequency in tests on tissue sections of the human and animal heart. More than 50% of the sera reacted with intercalary disks of the myocardial muscle fiber when tested on human and guinea-pig tissue sections. A reaction with these structures on bovine tissue sections was much less common. No sigificant differences were found in the frequency of detection of antibodies against smooth-muscle elements of the vessel wall.

In 20 of 38 cases when sera of rheumatic fever patients were tested on bovine heart tissue sections reactions were observed with interstitial connective-tissue cells (Fig. 1D). Some sera reacted with these same structures in sections through the guinea-pig heart, but the reaction was less clear. When the sera were tested on human heart tissues no luminescence of these elements was found.

When the sera was tested on different specimens of heart tissue taken from different individuals of the same species, identical results were obtained.

The reactions found are not specific for rheumatic fever, for antibodies against the same elements of the muscle fiber and interstitial connective-tissue cells have also been found in other diseases (chronic tonsillitis, idiopathic myocarditis). During the study of the donors' sera in some cases weak reactions were observed with elements of the muscle fiber and interstitial connective-tissue cells.

Antibodies against many components of the myocardial muscle fiber were thus detected in the sera of rheumatic fever patients. By contrast with the results obtained by Kaplan [8] and Zabriskie et al. [12, 13], in the present experiments reactions were found with muscle fiber disks. Such reactions were also observed by Zisel'son et al. [2] who used rabbit heart tissues. In addition, reactions were found with myocardial intercalary disks.

The discovery of reactions with the interstitial connective-tissue cells of the heart is of particularly great interest. In other investigations, as has been said already, despite the presence of bound γ-globulin in the patient's connective tissue, no circulating antibodies against connective-tissue elements were found in the sera.

When the sera was tested on the heart tissues of different species, the frequency of detection of reaction with the various elements of the myocardial muscle fiber varied. It is noteworthy that Shiokawa et al. [11] also observed differences in the reactions with elements of the myocardial muscle fiber when parallel tests were made of sera on sections of the human and rabbit heart.

The more frequent discovery of reactions with the sarcolemma of the muscle fiber when sera were tested on human heart tissue sections may be dependent on the nature of the method (more prolonged contact with the serum and labeled antibodies). Other differences reveal during testing of the sera on different tissues (for example, positive reactions with bovine and guinea-pig connective-tissue elements despite negative results on human tissues) may perhaps depend on certain special features of these tissues. Organ- or tissue-specific antigens which are common to the various species must be assumed to be located differently in the tissues. Another possibility which cannot be ruled out is a different localization of the antigenic determinants which are identical for the common antigens of the different species. Because of the difference in localization, the antibodies of these substances or the determinants may differ in their accessibility in the tissues of different species. Further investigations are necessary to confirm or refute this hypothesis.

LITERATURE CITED

1. T. A. Danilova, N. A. Borodiyuk, and G. A. Ugryumova, Byull. Éksperim. Biol. i Med., No. 4, 71 (1971).
2. A. D. Zisel'son, Yu. N. Zubzhitskii, and V. V. Yur'ev, Vopr. Revmat., No. 2, 38 (1970).
3. S. Avrameas and T. Ternynck, Immunochemistry, 6, 53 (1969).
4. J. S. Baumstark, R. J. Laffin, and W. A. Bardawil, Arch. Biochem., 108, 514 (1964).
5. D. C. Dumonde, Advances in Immunology, 5, 245 (1966).
6. E. V. Hess, C. W. Fink, A. Taranta, et al., J. Clin. Invest., 43, 886 (1964).
7. M. H. Kaplan and F. D. Dallenbach, J. Exp. Med., 113, 1 (1961).
8. M. H. Kaplan, M. Meyeserian, and J. Kushner, J. Exp. Med., 113, 17 (1961).
9. M. H. Kaplan and K. H. Svec, J. Exp. Med., 119, 651 (1964).
10. R. Lanningan and S. Zaki, Nature, 217, 173 (1968).
11. Y. Shiokawa, S. Yamada, and H. Shibayama, Jap. Heart J., 4, 407 (1963).
12. J. B. Zabriskie, Advances in Immunology, 7, 147 (1967).
13. J. B. Zabriskie, K. C. Hsu, and B. C. Seegal, Clin. Exp. Immunol., 7, 147 (1970).