ANTIBODIES AGAINST HETEROLOGOUS CONNECTIVE-TISSUE CELLS IN RHEUMATIC FEVER

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Antibodies against interstitial connective-tissue cells of the bovine heart in rheumatic fever and other diseases were studied by the immunofluorescence method. Adsorption studies showed that the antigen (antigens) to which these antibodies are opposed is (are) constantly present in the connective tissue of various bovine organs, including red cells. No identical antigen could be found in human connective tissue.

The pathological process in rheumatic fever is localized predominantly in the connective tissue. Nevertheless, antibodies against connective-tissue cells have virtually not been found in patients' sera [5, 15], despite the fact that deposits of bound γ globulin have been found in connective-tissue structures of the heart in rheumatic fever [4, 7]. It was shown previously [2] that the sera of patients with rheumatic fever, when tested by the indirect immunofluorescence method on sections of bovine heart tissue, react with the cells of the interstitial connective tissue.

This paper describes a further investigation of antibodies against connective-tissue cells in rheumatic fever and other diseases and also a study of the specificity of the reactions discovered in adsorption tests with the sera and homogenates of various bovine and human tissues and red cells.

EXPERIMENTAL METHOD

Investigations were carried out by the indirect immunofluorescence method using pure antibodies against human γ globulin prepared by the method of Avrameas and Ternynck [3] and labled with fluorescein isothiocyanate. The technique of preparing and labeling the pure antibodies was described previously [1, 2].

The sera of patients with rheumatic fever in the active (73 sera) and inactive period (42 sera) and also after the operation of transplantation of an artificial aortic valve (7 sera) were studied. Sera from patients with myocardial infarction (24 sera) and of healthy subjects (donors) as the control also were tested. Antibodies were studied on tissue sections of the bovine heart. The heart tissue was taken from the wall of the left ventricle of healthy animals. Some sera were tested on tissue sections of the human heart (biopsy material – tissue of the auricle obtained at commissurotomy on a patient with blood group O). The pieces of tissues were frozen in petroleum ether at −70°C. Sections 4 μ in thickness were cut in a cryostat at between −20 and −30°C and were used unfixed.

To adsorb the sera, tissue homogenates were prepared from various organs – the bovine and human heart, heart valves, liver, spleen, and pericardium. To prepare the homogenate the tissue was minced, an equal volume of 0.85% NaCl solution was added, and the mixture was homogenized at 4°C in a tissue microblender. The tissue residue was separated by centrifuging at 5,000-6,000 rpm at 4°C and washed in the cold with 0.85% NaCl solution 4 or 5 times until a translucent supernatant was obtained. The serum in a working dilution (1:4) was mixed with the tissue residue in the ratio of 2:1 and kept for 1 h at 37°C and 18 h at 4°C [6].

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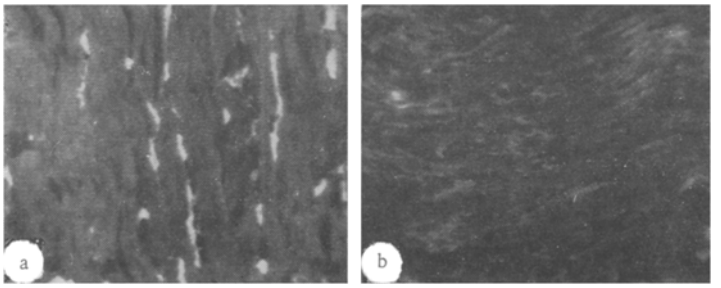

Fig. 1. Reaction of sera of patients with rheumatic fever with bovine heart tissue sections: a) fluorescence of interstitial connective-tissue cells; b) absence of fluorescence of interstitial connective-tissue cells when the same serum adsorbed by sheep's red cells was used.

Human red cells of all four groups (A, B, AB, O) and bovine and sheep red cells were used in the adsorption experiments. A suspension of washed red cells was added to the undiluted serum in the ratio of 4:1 and kept for 18 h at 4°C. After adsorption the sera were tested in a working dilution.

The method of treating the sections with sera and pure antibodies was described previously [2]. The preparations were examined with the ML-2 luminescence microscope using a 40 ×objective (water immersion) and homal 3 ×ocular. The intensity of the reaction was assessed by a system of plus signs.

EXPERIMENTAL RESULTS

When the sera of patients with rheumatic fever in the active and inactive period of the disease were tested on bovine heart tissue sections, reactions were regularly found with the cells of the interstitial connective tissue (Fig. 1a). In the active period of rheumatic fever intensive reactions (more than ++) were found considerably more often (in 40% of cases) than in the inactive period (11.4%). The frequency and intensity of these reactions were particularly high in patients with rheumatic fever after transplantation of an artificial valve (in six of seven cases). Antibodies against interstitial connective-tissue cells were also found in 50% of patients with myocardial infarction. These reactions are thus not specific for rheumatic fever. The sera of healthy donors did not react or reacted only weakly with the heart tissue sections.

When the various sera were tested on heart tissue sections from persons of blood group O no reactions were found with the interstitial connective-tissue cells.

The study of the specificity of the reactions found to bovine heart tissue sections was carried out by testing the adsorption of the sera with homogenates of various tissues and red cells and their subsequent testing on bovine heart tissue sections. Sera used in these tests were those whose reaction with connective-tissue cells of the heart was assessed as between ++ and ++++. The results of adsorption of the sera by homogenates of bovine organs and red cells are given in Table 1.

During adsorption with heart tissue homogenate in five cases the fluorescence of the interstitial connective-tissue cells was not completely abolished. The results of adsorption with liver tissue homogenate were inconsistent: in most cases (9 of 14) the fluorescence was not completely abolished but in three cases it was completely abolished. Similar results (incomplete abolition of the fluorescence) were obtained on adsorption of the sera with heart valve tissue homogenate. Homogenate of spleen tissue was much more active – in all five cases the fluorescence was abolished. Definite results also were obtained by adsorption with pericardial homogenate: in all cases the fluorescence of the connective tissue was completely abolished.

The fluorescence was abolished equally completely after adsorption of the sera with bovine red cells (12 cases; Fig. 1b). In some tests the red cells used were from the same animal as that from which the myocardium was taken. In other tests a mixture of red cells from several animals was used.

To detect the corresponding antigen in human tissues the same sera were adsorbed with tissue homogenates of various human organs – heart, liver, spleen, and pericardium – after which the sera were tested on bovine heart tissue sections. Similar results were obtained in all 16 cases – adsorption by human tissues did not affect the results of the reaction between the sera and the bovine connective-tissue cells. Human red cells (of any blood group) likewise did not abolish the fluorescence of the connective-tissue cells.

TABLE 1. Results of Adsorption of Sera of Patients with Rheumatic Fever and Other Diseases by Homogenates of Various Bovine Organs and Red Cells

Tissue take for adsorption of sera	No. of tests	Results of adsorption: fluorescence of cells partially removed	Results of adsorption: fluorescence of cells completely removed
Homogenate:			
of heart tissue	5	5	0
of liver tissue*	14	9	3
of heart valves	2	2	0
of spleen	5	0	5
of pericardium	6	0	6
Red cells	12	0	12

*On adsorption with liver tissue homogenate in two cases the fluorescence of the cells was completely preserved.

To study whether the reactions observed were connected with Forssman's antigen, the sera were adsorbed with sheep's red cells. In this case the fluorescence of the cells was completely preserved.

Antibodies against an antigen localized in the interstitial connective-tissue cells of the bovine heart were thus detected in human sera. This antigen is evidently common to various cells of bovine connective tissue – the interstitial tissue of the heart, pericardium, spleen, and red cells. This antigen is not an isoantigen, for it was constantly found in the heart tissues of all animals of this species tested. In addition, adsorption showed that this antigen is present in the red cells of all individuals studied.

These reactions were not connected with the presence of Forssman's antigen in the cells. Attempts to find the antigen in human tissues were unsuccessful. The sera did not react with connective-tissue cells from the heart of a person with blood group O (in whose tissues there are no blood group isoantigens). In addition, none of the tissues taken for adsorption, including red cells, abolished the fluorescence of the bovine connective-tissue cells.

In connection with the data described above, the reports of a number of workers to the effect that human and animal serum contains heteroantibodies, i.e., antibodies against tissues of other species of animals, are interesting. For instance, an increase in the titer of antibodies against rat red cells has been described in human sera after kidney transplantation [14]. Guinea pig serum contains natural cytotoxic antibodies against mouse thymus cells and, to a lesser degree, against rat thymus cells [12]. Cytotoxic antibodies against mouse lymphocytes have also been found in normal human, rabbit, and guinea pig serum; the cytotoxic effect of the serum rises sharply after treatment of the mouse cells with neuraminidase [11].

The problem of which antigens stimulate the formation of heteroantibodies in an animal of another species has not been studied. In some papers antibodies against "latent" determinants of tissues of the same species have been described. For instance, a whole series of antibodies evidently aimed at latent determinants of γ globulin have been found in human serum. Examples of antibodies of this type are rheumatoid factor [10], antibodies against the Fab-fragment [8], and antibodies against other concealed sites on the γ-globulin molecule [9].

The results of this investigation suggest that antigens stimulating the appearance of antibodies against bovine connective-tissue cells exist in the human body, for the intensity of the reactions rises considerably during activation of the pathological process (rheumatic fever, myocardial infarction) and also after transplantation of an artificial valve. Steffen et al. [13] demonstrated the presence of biochemically similar structures in species-specific determinants of bovine and human collagen. On this basis it can be postulated that the antibodies found in the present investigations are directed against common bovine and human antigenic determinants. Under normal conditions in man these common antigenic determinants of the connective tissues are evidently "latent." To discover whether "latent" antigenic determinants identical with the determinants of bovine connective tissue with which the sera of patients with rheumatic fever react are present in human connective tissue, further investigations must be carried out.

LITERATURE CITED

1. T. A. Danilova, N. A. Borodiyuk, and G. A. Ugryumova, Byull. Éksperim. Biol. i Med., No. 4, 71 (1971).
2. T. A. Danilova and I. M. Lyampert, Byull. Éksperim. Biol. i Med., No. 3, 68 (1972).
3. S. Avrameas and T. Ternynck, Immunochemistry, 6, 53 (1969).
4. M. H. Kaplan and F. D. Dallenbach, J. Exp. Med., 113, 1 (1961).
5. M. H. Kaplan, M. Meyeserian, and J. Kushner, J. Exp. Med., 113, 17 (1961).
6. M. H. Kaplan and K. H. Svec, J. Exp. Med., 119, 651 (1964).
7. R. Launigan and S. Zaki, Nature, 217, 173 (1968).

8. W. J. Mandy and L. C. Kormeier, Science, 154, 651 (1966).
9. C. L. McLaughlin and A. Solomon, Science, 179, 580 (1973).
10. F. Milgrom, Ann. New York Acad. Sci., 124, 118 (1965).
11. P. K. Ray and R. L. Simmons, Proc. Soc. Exp. Biol. (New York), 142, 217 (1973).
12. M. Schlesinger, Progr. Allergy, 16, 214 (1972).
13. C. Steffen, M. Dichtl, W. Knapp, et al., Immunology, 21, 649 (1971).
14. T. S. Tiong and P. J. Morris, Clin. Exp. Immunol., 10, 163 (1972).
15. J. B. Zabriskie, K. C. Hsu, and B. Seegal, Clin. Exp. Immunol., 7, 147 (1970).