A STUDY OF CROSS-REACTIONS BETWEEN STREPTOCOCCAL GROUP A ANTIGENS AND FIBROBLASTS OF CARDIAC INTERSTITIAL CONNECTIVE TISSUE FROM MAMMALS OF DIFFERENT SPECIES

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UDC 576.851.214.097.2:616.127-018.2-097.5

KEY WORDS: myocardium; fibroblasts; streptococcus; cross-reacting antigens and antibodies.

Reactions of sera from rabbits immunized with nontype-specific (NTS) group A streptococcal antigens with interstitial connective tissue (ICT) cells of human myocardium have been found by the indirect immunofluorescence method. On the basis of results of streptococcal adsorption experiments it has been suggested that there exists a cross-reacting (CR) antigen common both to the streptococcus and to fibroblasts of the human myocardium [5].

The object of the present investigation was to determine the corresponding CR-antigen in ICT of the myocardium from persons with different blood groups, in human embryos, and in mammals of different species, and also to look for CR-antigen in rabbits whose sera, after immunization with streptococcal NTS-antigens, react with ICT of human myocardium. These investigations are essential in order to decide whether the antibodies arising in response to the test CR-antigen are autoantibodies. Investigations on cell membranes of different tissues have revealed immunoglobulin receptors which react with the Fc-region of immunoglobulins [7, 10, 12]. To rule out the possibility of obtaining reactions of immunoglobulins with myocardial ICT on account of Fc-receptors, one aim of the investigation was to detect reactions with $F(ab')_2$ -fragments of IgG isolated from immune sera.

EXPERIMENTAL METHOD

Rabbits weighing 1.5-2 kg, whose sera did not react with the various structures of the human myocardium, were immunized by the method of Goudie et al. [8] with fractions containing NTS-antigens. The NTS-antigens were obtained by preparative electrophoresis from cultures of group A streptococci of types 1 and 29, grown on broth with casein hydrolysate [2]. The rabbits' sera were tested by the indirect immunofluorescence method (IIFM) with pure antibodies against rabbit IgG, labeled with fluorescein isothiocyanate. Unfixed heart tissue sections from 22 healthy persons dying from trauma at the age of between 6 and 25 years, from human embryos aged 18 weeks or over (three specimens), and also sections of ox, pig, guinea pig, and rat myocardium (three specimens from each species), from nonimmunized rabbits (six specimens), and from rabbits immunized with group A streptococcal NTS-antigens (seven specimens) were used. The methods of preparing the labeled antibodies and of obtaining and processing the tissue sections were described previously [2]. Sections of rabbit myocardium were first washed with buffered 0.85% NaCl solution. The sections were examined with the ML-2 luminescence microscope (40× objective). The homal 3 ocular was used for photography. Altogether 32 sera from rabbits immunized with group A streptococcal NTS-antigens and 111 normal rabbit sera were tested. To obtain $F(ab')_2$-fragments, preparations of IgG isolated from normal and immune sera were subjected to pepsin hydrolysis and fractionation by gel-filtration on a column with Sephadex G-150 [11]. Fractions containing $F(ab')_2$-fragments were concentrated by dialysis under pressure [6]. The purity of the preparations of $F(ab')_2$-

Laboratory of Streptococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi i Meditsiny, Vol. 89, No. 5, pp. 582-584, May, 1980. Original article submitted June 24, 1979.

 0007-4888/80/8905-0640$07.50

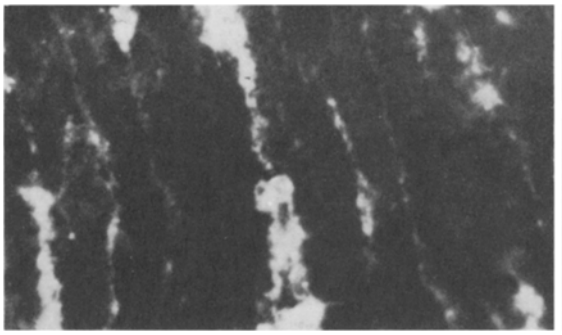

Fig. 1

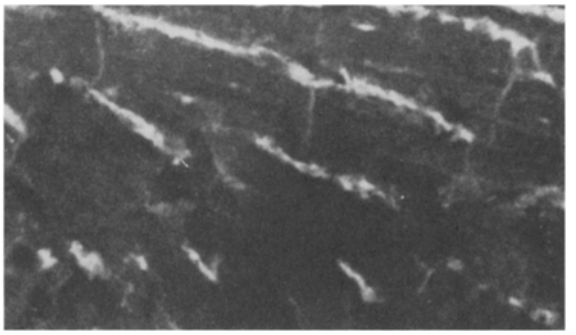

Fig. 2

Fig. 1. Reaction to labeled antibodies against rabbit IgG with myocardial ICT of a rabbit immunized with streptococcal NTS-antigens. Intensive fluorescence of all connective-tissue structures of the myocardium (after preliminary washing of section for 24 h). Magnification: objective 40, ocular homal 3.

Fig. 2. Testing by indirect immunofluorescence method of $F(ab')_2$-fragments isolated from serum of rabbit immunized with group A streptococcal antigens. Reaction with ICT cells of human myocardium. Magnification: objective 40, ocular homal 3.

fragments was assessed by the gel diffusion test with donkey serum against rabbit IgG.* A preparation of $F(ab')_2$-fragments obtained in the laboratory of the N. F. Gamaleya Institute of Epidemiology of Microbiology, Academy of Medical Sciences of the USSR, was used as the control.

EXPERIMENTAL RESULTS

Sera of rabbits immunized with streptococcal group A NTS-antigens, in dilutions of 1:8-1:64, reacted with ICT cells of human myocardium in 17 of 32 cases (53.1%). Positive reactions with ICT cells during tests of immune sera were obtained with all specimens of human myocardium irrespective of blood group, in sections of human embryonic myocardium, and also with all specimens of ox, pig, guinea pig, and rat myocardium. Sera of unimmunized rabbits in four (3.6%) of 111 cases gave a weak reaction with ICT cells of human myocardium in a dilution of 1:8. No positive reactions were found on sections of ox, pig, guinea pig, and rat myocardium with nine normal sera. Tests of labeled antibodies against rabbit IgG on sections of rabbit myocardium without preliminary washing revealed fluorescence of all connective-tissue structures of the myocardium. Washing with buffered 0.85% NaCl solution at pH 7.2 for 20 min removed this fluorescence practically completely from the myocardial sections from unimmunized rabbits. In sections through the myocardium on rabbits immunized with streptococcal group A NTS-antigens intensive fluorescence of the connective-tissue structures did not disappear even after washing with buffered 0.85% NaCl solution at pH 6.8 for 24 h (Fig. 1). In myocardial sections from unimmunized rabbits, washed for 20 min, when tested with sera reacting intensively with ICT cells from the myocardium of other species, virtually no positive reactions were found with ICT; in some cases only weakly positive reactions (±) were observed with sera in low dilutions (up to 1:8). Preliminary washing of tissue sections from the human heart under the same conditions had no effect on the intensity of fluorescence of ICT cells.

Testing preparations of $F(ab')_2$-fragments isolated from immune and normal sera in the gel-diffusion test with donkey serum against rabbit IgG revealed one precipitation band. These preparations gave partial identity with rabbit IgG (Serva) and complete identity with the control preparation of $F(ab')_2$-fragments. Preparations of $F(ab')_2$-fragments isolated from immune sera reacted in the IIFM with ICT cells of human myocardium and myocardium from other species of mammals except rabbits, in concentrations of 300 μg/ml and above (Fig. 2). No re-

*The serum was obtained from the Laboratory of Luminescent Diagnostic Sera of the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

actions of these same preparations of $F(ab')_2$-fragments with ICT cells could be found on sections of the rabbit myocardium. The $F(ab')_2$-fragments obtained from normal rabbit sera did not react with ICT cells from the myocardium of any species.

More than half of the sera of animals immunized with group A streptococcal NTA-antigens thus reacted intensively with myocardial ICT cells of man and other species of mammals (ox, pig, guinea pig, rat). The extreme rarity of positive reactions with myocardial ICT cells when normal rabbit sera were tested and the presence of positive reactions with $F(ab')_2$-fragments of IgG isolated from immune sera are proof of the specificity of these reactions. CR-antigen found in the ICT cells is evidently a tissue- or organ-specific antigen such as are found in all individuals of different species. It is to CR-antigens such as these that autoimmune reactions usually arise [4]. Meanwhile antibodies against CR-antigen of myocardial ICT cells cannot be classed as autoantibodies, for the question of whether ICT of the rabbit myocardium contains true CR-antigen is not yet settled. The absence of reactions on sections of rabbit myocardium may be due to the low content of CR-antigen in ICT of the rabbit myocardium or it could be explained on the grounds that CR-antigen is a "latent" antigenic determinant. That is possibly why rabbits are good producers of antibodies against the CR-antigen now being studied, which react with myocardial ICT cells of other species of mammals. Washing sections of the rabbit myocardium to remove serum proteins with which ICT is impregnated [3] does not, evidently, lead to a decrease in the quantity of CR-antigen, for under these same conditions the intensity of the reactions with human ICT was substantially unchanged. The discovery of bound immunoglobulins in myocardial ICT of immunized rabbits is evidently proof of antibody productions against ICT antigens in the immunized animals. The problem of whether circulating antibodies reacting with ICT of heterologous myocardium and antigens bound with homologous rabbit ICT are directed against the same or different antigens of myocardial ICT cells remains unanswered.

In rheumatic fever [9], and also in animals immunized with homologous heart tissue mixed with a culture of streptococcus [1], a similar phenomenon has been described. In both cases bound immunoglobulins were found in ICT, but under these circumstances no circulating antibodies to homologous ICT were discovered. Meanwhile the sera of patients with rheumatic fever react in a high percentage of cases with ICT of bovine myocardium [2], i.e., in a heterologous system. Immunization with group A streptococcal NTS-antigens can evidently be used as a model for the study of the cause of such disagreements. Moreover, a further study of CR-antigen found in fractions containing group A streptococcal NTS-antigens is essential, for this antigen may be used to search for antibodies against myocardial ICT antigens in rheumatic fever.

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