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MONOCLONAL ANTIBODIES CROSS-REACTING WITH FIBROBLASTS OF MYOCARDIAL INTERSTITIAL CONNECTIVE TISSUE AND WITH GROUP A STREPTOCOCCAL CELL WALL PROTEIN ANTIGENS

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A cross-reacting antigen (CRA) was found previously among the nontype-specific antigens (NTSA) of the cell wall of the group A streptococcus [5]. Sera of rabbits immunized with NTSA, when tested by the indirect immunofluorescence method (IIFM), have been shown to react with myocardial interstitial connective tissue (ICT) cells and with cultures of fibroblasts [5]. Deposits of bound immunoglobulins have been discovered in the myocardial ICT of immunized animals. In spite of this, only heterophilic antibodies, reacting with ICT of other species of mammals, and not autoantibodies, have been found in the sera of these animals. The reasons why bound immunoglobulins are found in the myocardial ICT in spite of the absence of autoantibodies in the sera is not yet understood. The solution to this problem is interesting because a similar phenomenon, namely the discovery of heterophilic antibodies, and not of autoantibodies, in sera and of bound immunoglobulins in the myocardial ICT, has been described in rheumatic fever [6, 9].

The aim of this investigation was to obtain monoclonal antibodies (McAb) to cell wall NTSA of the group A streptococcus, cross-reacting with myocardial ICT cells and with fibroblasts of other mammalian organs, and also to examine the question of whether these antibodies are autoantibodies or not.

EXPERIMENTAL METHOD

BALB/c mice were immunized with a fraction containing a streptococcal cell wall NTSA. Cultures of group A streptococcus with enhanced virulence were used (type 5M 20/59 and type 29M 15/55, Prague Collection, obtained from Dr. J. Rotta, Czechoslovakia). The cultures were grown on medium with casein hydrolysate without addition of serum and were washed off 3 times with 0.85% NaCl solution, pH 7.2. HCl extracts were prepared by Lancefield's method. The NTSA fraction was isolated from the HCl extracts by preparative electrophoresis [10]. The fraction obtained from type 5 streptococci was injected intraperitoneally in a dose of 20 μg 8 times at intervals of 7 days. The last injection was given 3-4 days before removal of the spleen. The first dose of the fraction was injected together with Freund's complete adjuvant (FCA). Hybridomas and McAb were obtained by the usual method [12] as described previously [1]. On fusion of the splenocytes with a myeloma of strain SP-2/0, 50% polyethylene-glycol (mol. wt. 4000, "Serva") was used. To prepare the ascites fluids, 10 days after injection of 0.5 ml of pristane ("Sigma") the mice were given an intraperitoneal injection of 10^7 hybridoma cells. Supernatants were screened and McAb studied by the ELISA method as described previously [4]. Peroxidase-labeled rabbit antibodies to mouse immunoglobulins were used [the antibodies were obtained from the Laboratory of Immunologic Diagnosis (Director K. D. Shakhanina), N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR].

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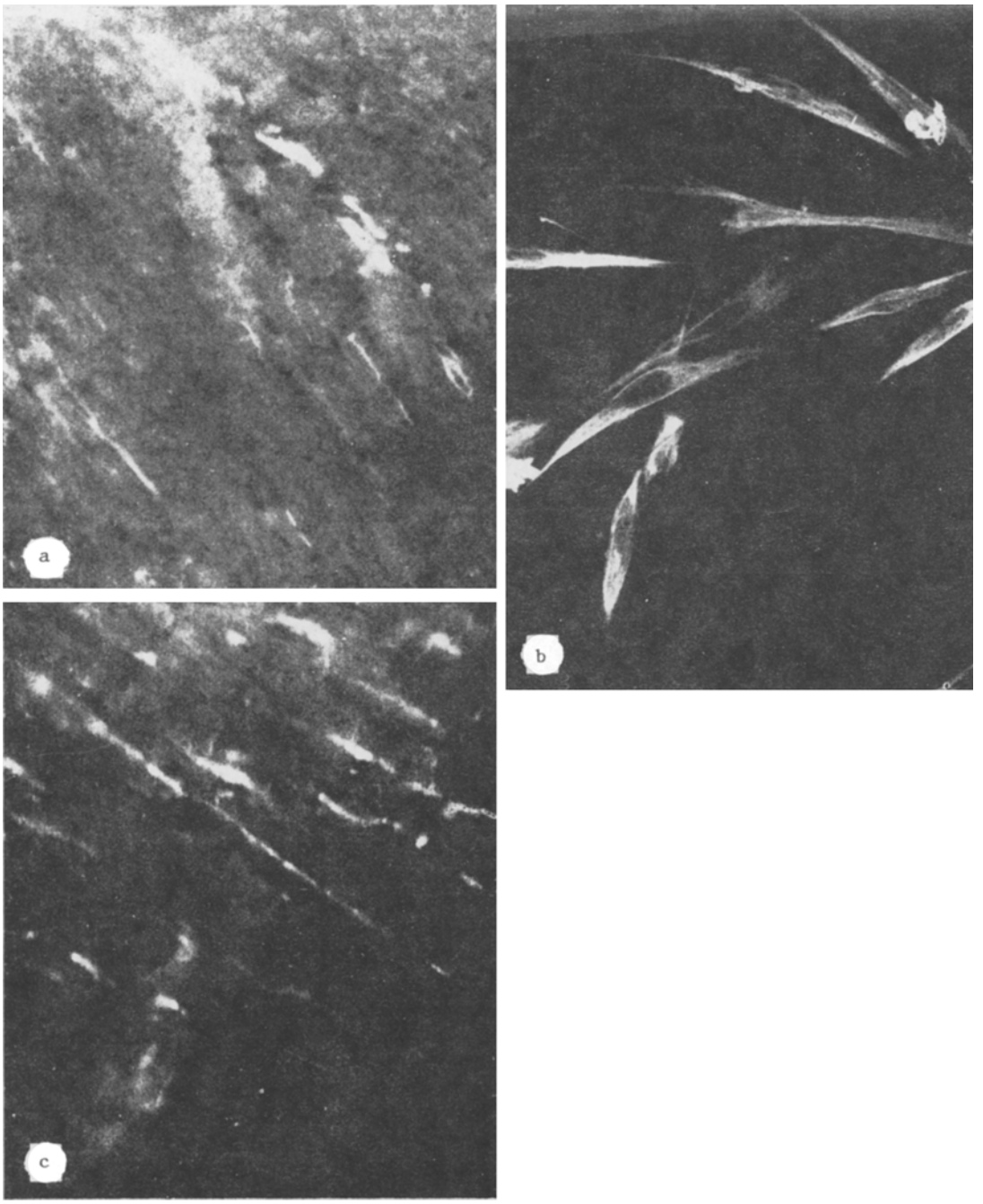

Fig. 1. Reaction of B6/5 McAb with myocardial interstitial connective tissue (ICT) cells on section of bovine heart (a), with fibroblasts on a fixed monolayer culture of human skin fibroblasts (b), and reaction of labeled antibodies to mouse immunoglobulins with bound immunoglobulins in myocardial ICT on sections through myocardium of BALB/c mouse (c).

The NTSA fractions or HCl extracts obtained from streptococci of types 5 and 29 (10 μg/ml) were applied to 96-well panels (USSR origin) in 0.05 M carbonate buffer, pH 9.6. Whole heat-killed streptococcal cells of types 5 and 29 or a culture of group A streptococcus treated with pepsin [13] also was used. Orthophenylenediamine was used as the substrate. The reaction was read on a "Titertek" photometer. The supernatants of the hybridomas were screened and McAb studied in parallel tests on unfixed frozen tissue sections of human fetal joints, sections through skin and heart of BALB/c mice, and human, rabbit, and bovine heart tissues. Tests also were carried out on monolayer cultures of fibroblasts explanted from human skin. The cultures were grown for 3-4 days on coverslips and fixed with acetone [2]. In some experiments the fibroblasts were treated with colchicine [8]. The IIFM was used as described previously [11]

with antibodies to mouse immunoglobulins, labeled with fluorescein isothiocyanate. To determine bound immunoglobulins in the myocardial ICT the direct immunofluorescence method [9] was used. The reactions were read on the ML-2 luminescence microscope with 40× objective. A Homal 3× objective was used for photography. Reactions of McAb with the tissues were inhibited by the use of the NTSA fraction, HCl extracts of streptococci of types 5 and 29, BCG, and also a culture of group A streptococcus, heat-killed or treated with pepsin. Supernatants (in a dilution of 1:2) obtained during growth of the monoclones, were adsorbed in the proportion of 40 mg protein or dry weight to 1 ml. A6/1 McAb, obtained previously and reacting with the basal layer of the skin epithelium and not with streptococcal antigens [1], were used as the control.

EXPERIMENTAL RESULTS

Investigation of the serum of a mouse immunized with NTSA fraction by the IIFM reveal a very weak reaction in myocardial sections of a BALB/c mouse with myocardial ICT cells, but a stronger reaction with bovine ICT. On primary screening of supernatants of the clones by the ELISA method positive reactions with the NTSA fraction were obtained in 91 (35%) of 254 cases. During testing of the supernatants of 91 clones by the IIFM, antibodies reacting with human and bovine myocardial ICT cells were found in 16.4% of cases. As a result of further cloning, monoclone B6/5 producing McAb giving intensive fluorescence of the cytoplasmic membrane of myocardial ICT cells, morphologically similar to fibroblasts (Fig. 1a), was obtained. The B6/5 McAb induced fluorescence of cells morphologically similar to fibroblasts on sections of the myocardium, in the loose connective tissue surrounding blood vessels, and also on sections through human embryonic heart valves and joints. On testing the McAb on cultures of human fibroblasts, an intense reaction was observed with the intermediate filaments, running parallel to the long axis of the fibroblasts. No reaction was observed with the cell nuclei (Fig. 1b). It was shown by the IIFM that another monoclone (C5/3) synthesizes McAb reacting with discs of myocardial muscle fibers and the skeletal musculature. No reactions were found to cultures of fibroblasts, and also with myocardial ICT cells when tested with C5/3 McAb and the control A6/1 McAb. Positive reactions with the corresponding structures were observed on myocardial sections from BALB/c mice and from the human, rabbit, and bovine heart, when tested with B6/5 and C5/3 McAb. Positive reactions also were obtained in the ELISA test with the NTSA fraction (types 5 and 29) or with a whole culture of group A streptococcus, when tested with B6/5 and C5/3 McAb. No reactions were found on testing B6/5 and C5/3 McAb on a streptococcal culture treated with pepsin. Total inhibition of reactions of B6/5 McAb with fibroblasts and C5/3 McAb with disks of myocardial muscle fibers was obtained with the aid of the NTSA fraction and HCl extracts of group A streptococcus of types 5 and 29. Adsorption with a streptococcal culture treated with pepsin, in which no NTS streptococcal group A cell wall proteins were present, or with BCG, did not affect the intensity of the reactions of B6/5 and C5/3 McAb with the tissue structures. No inhibition of reactions of the control A6/1 McAb with cells of the basal layer of the skin epithelium was found when whole streptococcal cells and preparations containing NTSA were used.

To determine bound immunoglobulins in myocardial ICT, myocardial tissues of four mice were studied after culture of hybrid B6/5 cells, producing McAb to myocardial ICT cells, in the peritoneal cavity of these mice for 2 weeks. Deposits of bound immunoglobulins were found in the myocardial ICT of these mice by the direct immunofluorescence method (Fig. 1c). No bound immunoglobulins were found in the ICT of three normal BALB/c mice and of two mice after culture of hybrid cells of the C5/3 clone. Fixation of C5/3 McAb in muscle tissue likewise was not found. Thus the B6/5 McAb are evidently antibodies to streptococcal CRA, for agreement was obtained between the reaction with connective tissue and the positive results of the ELISA test with group A streptococcal NTSA. In addition, complete inhibition of reactions of B6/5 McAb with fibroblasts was discovered when NTSA obtained from streptococci of types 5 and 29 were used. Inhibition of reactions of B6/5 McAb was not found in the ELISA test in experiments with adsorption by a culture of group A streptococcus treated with pepsin and containing group polysaccharide from the surface. These data are evidence that a CRA common with NTSA of group A streptococcus is present in cultures of fibroblasts and in myocardial ICT. The CRA found in fibroblasts is evidently related to vimentin. Typical redistribution and compression of filaments with which B6/5 McAb react was found in the perinuclear zone in a culture of fibroblasts treated with colchicine. This, as we know, is characteristic of intermediate filaments of vimentin type [8].

According to data in the literature, some determinants of streptococcal M protein can fix fibrinogen [14]. The NTSA fractions may contain M protein, but they probably do not contain

fibrinogen, because the streptococcal cultures were grown on casein medium without the addition of serum and were thoroughly washed. During immunization with NTSA, fixation of fibrinogen in an autologous system is evidently possible. The possibility cannot be ruled out that conjugation of fibrinogen with streptococcal antigens can lead to the production of antibodies to fibrinogen. Possibly these antibodies may belong to the heterophilic antibodies which are found in sera after immunization with NTSA fractions [5]. This problem requires further study. Absence or a low level of autoantibodies in the sera after immunization with NTSA may be connected with fixation of these antibodies on tissues. Inhibition of reactions of B6/5 McAb with myocardial ICT and fibroblasts with the aid of NTSA fractions, in which there is evidently no fibrinogen, is one confirmation of the presence of a CRA among the streptococcal antigens used for immunization. Since no Fc-receptors are found on fibroblasts of myocardial ICT [3], the reactions of McAb with fibroblasts of ICT described above are immunologically specific.

Thus direct proof of autoantibody production to fibroblasts on immunization with NTSA was obtained for the first time by a study of McAb and fixed immunoglobulins in the myocardial ICT of mice with ascites. These data explain why bound immunoglobulins are found in myocardial ICT — a phenomenon previously described in rheumatic fever [9]. According to other data [7], McAb obtained by immunizing BALB/c mice with streptococcal membrane antigens react not only with the cytoskeleton of human fibroblasts, but also with cell nuclei. Ability of McAb to be fixed in myocardial ICT was not determined under these circumstances, nor was it proved that they belonged to autoantibodies, since the tests were not carried out in a syngeneic system.

The experiments showed that C5/3 McAb are not fixed, not only in ICT, but also in the myocardial muscle tissue, with which they react on sections. Subsequent determination of fixation of various autoantibodies with tissues in the whole organism is thus evidently an indicator of the role of these autoantibodies in the autoimmune process.

B6/5 McAb must be used to isolate the corresponding CRA from the NTSA fraction and from fibroblasts to enable detailed immunochemical characterization of this antigen and to determine CRA in cultures of streptoccal group A isolated in rheumatic fever and other streptococcal infections, for the presence of the CRA may be an indicator of "rheumatogenic" strains.

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