MONOCLONAL ANTIBODIES TO GROUP A STREPTOCOCCAL POLYSACCHARIDE, CROSS-REACTING WITH MAMMALIAN CONNECTIVE TISSUE

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Investigations have shown that antibodies to group A streptococcal polysaccharide (A-PS) cross-react with the epithelium of the cortex and medulla of the thymus and with the basal layer of the epithelium of human and animal skin [3, 12]. Antibodies reacting with epithelium are autoantibodies. These results are confirmed by the obtaining of monoclonal antibodies (MCA) to A-PS, which react with the same epithelial structures of the skin and thymus [4].

Using polyclonal antibodies to A-PS, some investigators have found cross-reactions with connective tissue [10]. In other investigations these findings were not confirmed [12]. The possibility cannot be ruled out that sera or fractions tested by different investigators contained antibodies to different determinants of A-PS.

The aim of this investigation was to obtain MCA to A-PS and to test these antibodies on connective-tissue structures and, in parallel experiments, on mammalian epithelial tissues.

EXPERIMENTAL METHODS

Strains of streptococcus of groups A, L, and C (obtained from Dr. J. Rotta, Prague), and of an A variant (obtained from M. McCarty, USA) were used. The PS preparations were obtained by the formamide method from pepsin-treated microbial cells of streptococci of group A (A-PS), C (C-PS), L (L-PS), and A variant (V-PS) [8]. BALB/c mice (weighing 17-20 g) were immunized with a culture of group A streptococcus (strain J-17A4), treated with pepsin [6]. During the cycle, four times at intervals of 7 days, the culture was injected intraperitoneally in a dose of 20-50 μg rhamnose per mouse. The quantity of rhamnose was determined by Dische's method. Two cycles of immunization were given with an interval of 3 months. The mouse splenocytes were taken 3 days after the last immunization for fusion with NP plasmacytoma cells. Hybridization and obtaining of ascites fluids containing MCA were carried out by the usual methods [11] as described previously [2]. The clones were screened and the supernatants and ascites fluids containing MCA were tested by enzyme immunoassay (EIA), and by the indirect immunofluorescence method (IIFM). To perform EIA, plates were covered with cultures of streptococci of groups A, C, L, and A-variant, treated with pepsin, at the rate of $250 \cdot 10^6$ microbial cells to 1 ml in phosphate-buffered solution (pH 7.4). The test was carried out by the usual method [13]. Orthophenylenediamine was used as the substrate. Antibodies to mouse Ig, labeled with peroxidase, were used. For tissue assays, IIFM was used [12]. Unfixed frozen sections of the following tissues were studied: skin of the lip of BALB/c mice, human skin (embryonic joint), human, mouse, and bovine myocardium. Cultures of human fibroblasts, explanted from the skin of donors or patients with rheumatic fever also were used [1]. Antibodies to mouse Ig labeled with fluorescein isothiocyanate, in a dilution of 1:16, were used for IIFM. The reaction was read on the ML-2 luminescence microscope with 40× objective. A homal 3× ocular was used for photography. To inhibit the reaction of MCA with tissue structures, 10-17.5 mg of the different PS was added to 1 ml of superna-

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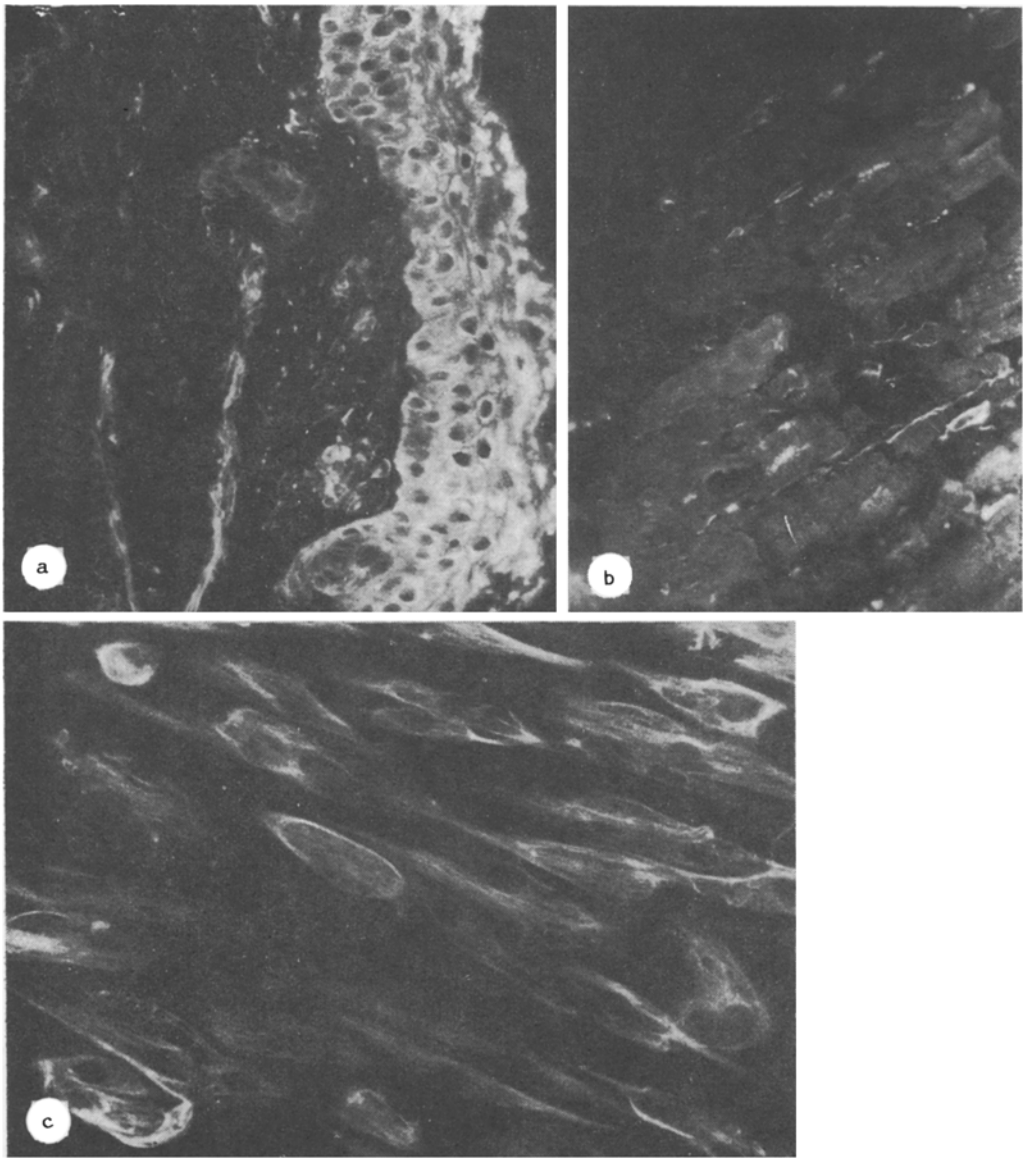

Fig. 1. Reactions of D4/1 MCA: a) with all layers of skin epithelium and with connective-tissue structures in sections through human embryonic skin; b) with ICT of myocardium in section through bovine heart; c) with fibroblasts on fixed monolayer culture of human fibroblasts.

tant or ascites fluid in a dilution of 1:8 or higher. For inhibition of reactions of MCA with streptococcal cultures in EIA, 500 μg of PS was added to 1 ml of supernatant or ascites fluid in a dilution of 1:120 or above. For comparative analysis, we used A3/2 MCA, which are directed toward a determinant common for PS of the A and L groups, and do not react with mammalian tissues [2]. The MCA were tested by immunodiffusion with sera to mouse IgG and IgM (Miles, USA).

EXPERIMENTAL RESULTS

Screening of the hybrid clones by EIA showed that 224 of the 400 clones gave positive reactions with a pepsin-treated culture of group A streptococcus. Of 54 clones tested by IIFM, reactions with different structures from human skin sections were found in 31 cases. For subsequent analysis and cloning a D4/1 hybridoma was used. D4/1 MCA induced fluorescence of all layers of the epithelium and of connective-tissue structures in sections of embryonic joint.

It was found that D4/1 MCA contained in ascites fluids react in titers of 1:12,800-1:25,000 in EIA with pepsin-treated cultures of streptococcus of groups A, C, L, and A-variant. Meanwhile A3/2 MCA reacted in high titers with cultures of streptococci of groups A and L, and there was virtually no reaction with cultures of C and A-variant.

Inhibition experiments showed that A, C, and L PS considerably inhibited the reaction of D4/1 MCA when tested in EIA. Under these circumstances complete inhibition was found only with V-PS. The reaction of MCA of clone A3/2 in EIA was inhibited only by A-PS and L-PS.

To determine cross reactions between A-PS and epithelial tissues of the skin and mammalian connective tissue, D4/1 MCA were tested by IIFM in dilutions of 1:2 up to 1:320 on sections of skin and myocardium.

The results showed that MCA produced by clone D4/1 (38 monoclones obtained after two to four reclonings were tested) induce fluorescence of all layers of the epithelium of human embryonic skin, and also react with connective-tissue structures in skin sections (Fig. 1a). MCA of clone A3/2 did not induce fluorescence in the tissues. Testing D4/1 on the skin of BALB/c mice revealed positive reactions with the corresponding structures. It was also shown that D4/1 MCA react with interstitial connective tissue (ICT) of human, murine, and bovine myocardium (Fig. 1b). D4/1 MCA were found to give identical reactions when tested on cultures of fibroblasts explanted from the skin of healthy donors or patients with rheumatic fever (11 monoclones were tested), whereas A3/2 MCA did not react with these cells (Fig. 1c).

Reactions of D4/1 MCA with epithelial cells and with a culture of fibroblasts partially inhibited all PS. Complete inhibition of fluorescence was observed only with V-PS.

The results obtained with inhibition by PS of reactions of D4/1 MCA with a culture of group A streptococcus and also in tests by IIFM on tissues are thus evidence that V-PS inhibit these reactions most intensively. The results suggest that D4/1 MCA react with a rhamnose determinant common for PS of different groups of streptococci. We know that V-PS consists only of rhamnose residues [7]. Consequently, this determinant is evidently more accessible for antibodies in V-PS. Since MCA were tested and EIA carried out on pepsin-treated cultures of streptococci, the participation of cell-wall protein antigens in these reactions was ruled out. Complete inhibition of the reactions by purified V-PS suggests that these antibodies evidently react with PS and not with a peptidoglycan. According to some data [14], cell-wall proteins of group A streptococcus can fix fibrinogen. Since in the present case the animals were immunized with a pepsin-treated streptococcal culture, the participation of fibrinogen when obtaining D4/1 MCA can be ruled out.

D4/1 MCA are autoantibodies, for they induce fluorescence of the analogous tissue structures in BALB/c mice. It was shown that D4/1 MCA belong to the IgG_1 class.

As a result of long-term immunization with a pepsin-treated culture of group A streptococcus, D4/1 MCA reacting with all layers of the epidermis, and also with ICT of the human and animal myocardium and with a culture of human fibroblasts, were obtained for the first time. It must be assumed that these MCA are directed toward a cross-reacting tissue antigen, a tissue antigen common to fibroblasts, epithelium, and the rhamnose determinant of A-PS. These experiments confirm results obtained previously with the aid of whole sera, relating to cross reactions between A-PS and connective tissue [10].

It must be emphasized that cross reactions between various tissue substrates were discovered in some investigations with MCA: for example, between DNA and cardiolipin, and also between DNA and actin, tubulin, and thyroglobulin [5, 9]. The narrow specificity of MCA and their orientation toward particular antigenic determinants evidently enables cross reactions to be discovered between different tissue substances.

It will be noted that D4/1 MCA are evidently directed toward a different A-PS determinant compared with the A6/1-D MCA obtained previously [4]. A6/1-D MCA were shown also to react with A-PS and V-PS. Meanwhile, when these MCA were tested in IIFM, reactions were found only with the basal epithelium of the skin. No reaction was found with the other layers of the epithelium or with connective tissue [4]. The rhamnose region V-PS has been shown to contain two antigenic determinants common for PS of different groups of streptococci [7]. It must accordingly be postulated that D4/1 and A6/1-D MCA are evidently directed toward different A-PS determinants. This is a problem for future study.

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T_8-POSITIVE LYMPHOCYTES IN INFLAMMATORY INFILTRATES OF THE VESSEL WALL IN NONSPECIFIC AORTO-ARTERITIS

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Nonspecific aorto-arteritis (NAA) is a systemic disease of inflammatory genesis affecting the aorta and main arteries, but intramural vessels are not involved [1]. The disease was described quite a long time ago [11], but its etiology and pathogenesis have not yet been explained. The possibility of the autoimmune, inflammatory-allergic, infectious, and genetic nature of NAA has been discussed [4]. Morphologically, fibrosis of the adventitia, damage to the elastic carcase of the tunica media, local thickening of the intima, and infiltration of the pathological focus chiefly by mononuclear cells are found in the wall of the affected vessels [1, 10]. The morphology of the vessels in NAA has so far been studied by the usual histological methods. Methods of immunomorphology and, in particular, those using monoclonal antibodies (McAb), which enable the types of cells involved in the pathological process to be accurately characterized, have now become widely adopted.

In the investigation described below the walls of blood vessels affected by NAA were studied by means of McAb to functionally different antigens of lymphocytes, and also polyclonal antibodies (PcAb) to basement membrane proteins, the presence of which is characteristic of vascular smooth-muscle cells (SMC).

EXPERIMENTAL METHODS

Segments of vessels removed at operations for NAA from 11 patients were investigated (details of the patients are given in Table 1). Specimens of vessels were frozen and kept in liquid nitrogen. Frozen sections 5 μ thick were dried at 20°C and stored at −20°C. Immediately before staining the sections were fixed in acetone at 20°C for 10 min, washed with phosphate-buffered saline (PBS; pH 7.4) and incubated with a solution of the first antibodies in a working dilution. As the first antibodies we used the following McAb: 1) LT_4 and LT_8

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