Monoclonal antibodies that distinguish between human aorta smooth muscle and endothelial cells

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A monoclonal antibody has been generated that interacts with the surface of cultured human aorta smooth muscle cells and does not bind to the endothelial cells from aorta and umbilical vein. An antigen recognized by the antibody has a molecular mass of 330 kDa as determined by electrophoresis of immunoprecipitate in SDS-polyacrylamide gel. The same antigen appeared to be present on the fibroblast surface while neither immunofluorescence, flow cytofluorimetry nor immunoprecipitation reveal it on the endothelial cell surface or in the Triton X-100 extract.

Smooth muscle cell *Endothelial cell* *Surface antigen* *Monoclonal antibody*

1. INTRODUCTION

Smooth muscle cells represent a large part of the cell population in the vascular wall. Abnormal proliferation and metabolic activity of these cells may be important in the pathogenesis of atherosclerosis [1–3]. Cultivation of smooth muscle cells is widely used as an approach to the study of metabolism and elements of contractile activity, as these cultured cells retain many of the biochemical properties characteristic of smooth muscle cells in the intact tissue [4–7]. However, vascular smooth muscle cells growing in culture are 'phenotypically modulated' according to certain criteria [8,9]. In addition to increased proliferative and biosynthetic activities they exhibit definite changes in the cytoskeleton and contractile apparatus: (a) desmin – the main compound of intermediate filaments in muscle cells [10] is removed by vimentin; (b) decreased amounts of both α-actin and smooth muscle myosin are found in cultured smooth muscle cells of vascular origin [11–13]. These in vitro changes may be relevant to the process of in vivo phenotypic modulation during certain pathological processes such as atherosclerosis. Since practically all known markers of smooth muscle cells are compounds of cytoskeleton and contractile apparatus [12–14] they may not be revealed in 'modulated cells' in culture.

In this report we present development and characterization of a monoclonal antibody that reacts with an antigen retained on the surface of cultured smooth muscle cells. The antigen has a molecular mass of 330 kDa and is localized on the surface of human aorta smooth muscle cells. Endothelial cells do not contain the antigen as was shown by immunofluorescence, immunoprecipitation and flow cytofluorimetry.

2. MATERIALS AND METHODS

The culture of smooth muscle cells from tunica media of human aorta was obtained according to Ross [15]. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10–15% human serum and 2–5% cell free ascitic fluid obtained from the patient suffering

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from heart failure. Endothelial cells from human umbilical vein (primary culture) and from human aorta (7th passage) were a generous gift of Dr S. Danilov (USSR Cardiology Research Center). Human smooth muscle cells of 6–8 passages were used for immunization procedures carried out as described in [16]. The first immunization was performed intraperitoneally with 10^6 cells per mouse. Hybridomas were obtained according to Köhler and Milstein [17]. Primary screening was performed by indirect immunofluorescence on living smooth muscle cells of 6–8 passages. Hybridoma supernatants were incubated with target cells for 30 min at 37°C in CO_2-incubator. Fluorescein isothiocyanate (FITC) labeled antimouse IgG (Miles Scientific) diluted 1–40 were used as the second layer antibodies. The washes were done with DMEM supplemented with 10% calf serum. The cells were postfixed in 4% formaldehyde prepared on phosphate buffered saline (PBS), mounted in 75% glycerol on PBS and observed with a Zeiss Photomicroscope III. Selected

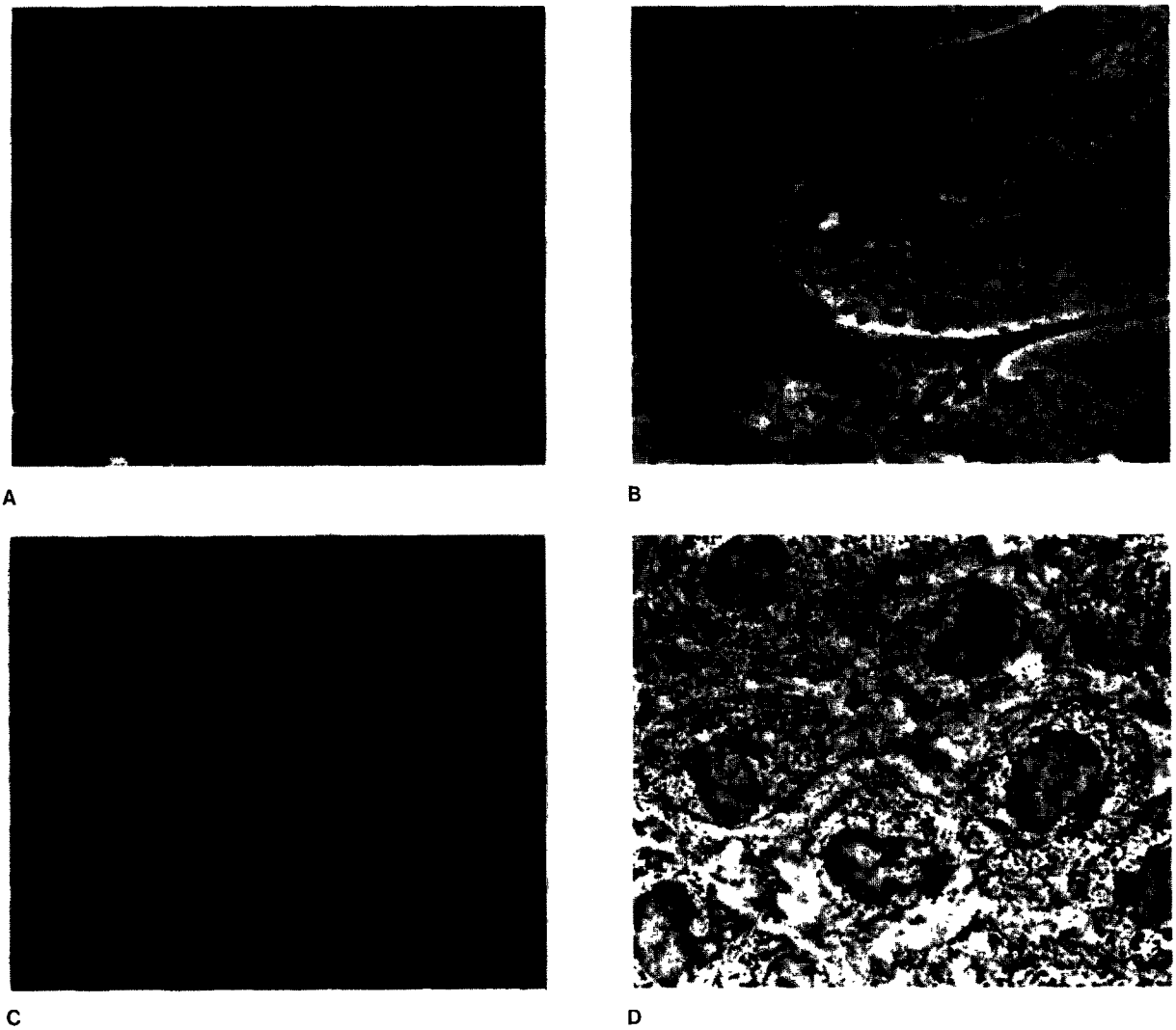

Fig.1. Immunofluorescent staining of living smooth muscle cells from human aorta with monoclonal antibody IIG10. (a,b) Human aorta smooth muscle cells, (c,d) human umbilical vein endothelial cells, (a,c) immunofluorescence, (b,d) phase contrast.

hybridomas were cloned by limiting dilution in 96-well culture plates. To obtain ascites about 10^7 cells per mouse were injected intraperitoneally. In 2–3 weeks ascitic fluid was collected and immunoglobulins were precipitated by $(NH_4)_2SO_4$ (50% saturation). Precipitates were dissolved in 0.2 M Na-borate buffer (pH 8.3) with 0.14 M NaCl, exhaustively dialyzed against the same buffer and stored frozen at −20°C. For flow cytofluorimetry, the cells were trypsinized gently and stained in suspension indirectly the same way as for immunofluorescence except that the incubations and washes were performed at room temperature. The last 3 washes were done with serum and indicator free medium and the postfixation step was omitted. Fluorescence was registered with a FACS II (Becton Dickinson, USA) cytofluorimeter. The wavelength of excitation light was 488 nm. For antigen identification the cells were labeled with [^{35}S]methionine (10 μCi/ml) for 18 h and then extracted in 1% Triton X-100 in 25 mM Tris-HCl buffer (pH 7.5, at 20°C) with 3 mM EDTA, 2 mM EGTA and 0.5 mM PMSF for 15 min at 4°C. To the clarified extract 50 μg/ml of primary (specific) antibodies were added together with gelatin up to 0.1% and NaCl up to 0.14 M. After 4 h incubation at 4°C, antibodies raised in rabbits against light chains of mouse immunoglobulins were added and incubated at 4°C for another 2 h. Finally, 200 μl of a 10% suspension of *Staphylococcus aureus* were added. After 1 h incubation at room temperature the precipitate formed was separated by centrifugation and analyzed electrophoretically [18].

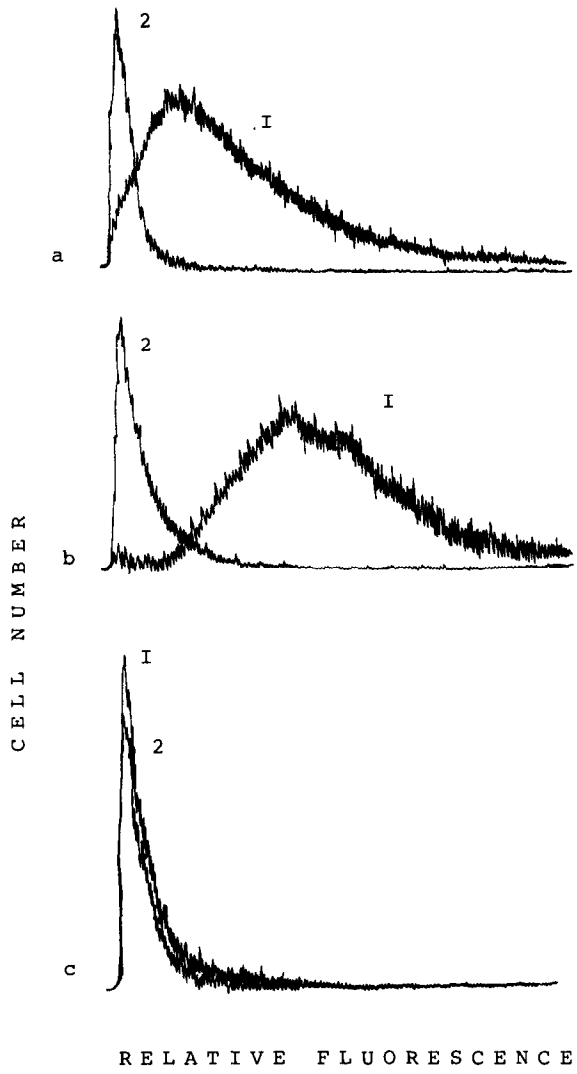

Fig.2. Flow cytofluorimetric analysis of the interaction of IIG10 antibody with different cells. Smooth muscle cells from human aorta, 7th passage (a), human skin fibroblasts, 4th passage (b) or endothelial cells from human aorta, 7th passage (c) were incubated in suspension with 50 μg/ml of antibody IIG10 (1) or nonimmune mouse immunoglobulins (2) followed by FITC-labeled secondary antibody (see section 2).

3. RESULTS AND DISCUSSION

To obtain antibodies to antigen specific for the smooth muscle cell surface mice were immunized with cultivated smooth muscle cells from media of human aorta. Specificity of the antibodies secreted by hybridoma was determined by indirect immunofluorescence technique. At first, the interaction of hybridoma supernatants with human smooth muscle cells was checked and then only those positive in this test were further examined on human skin fibroblasts and human umbilical cord endothelial cells. Culture supernatant from the well marked IIG10 appeared to contain antibodies reacting with the surface of living smooth muscle cells (fig.1) and fibroblasts (not shown), while endothelial cells were not stained at all. The primary population was cloned, subcloned, stable clones were selected and propagated further in vivo. Immunoglobulins (IgM, as determined serologically) obtained from the ascitic fluid exhibited the same

specificity as the culture supernatant. Reactivity of the IIG10 antibody with different cell types was examined by means of flow cytofluorimetry. The data presented in fig.2 show that antibody IIG10 reacts with smooth muscle cells, and fibroblasts as well, but does not bind to endothelial cells from human aorta. In the latter case, relative fluorescence activity does not exceed nonspecific background binding of the second layer FITC-labeled antibodies to target cells. Antigen recognized by IIG10 antibody was identified by immunoprecipitation. Fig.3 shows that immunoprecipitate obtained from Triton X-100 extract of smooth muscle cells in the presence of IIG10 antibody contains a polypeptide of 330 kDa. The antigen molecule may contain lipid or carbohydrate constituents that would affect SDS binding and electrophoretic mobility. Thus, the molecular mass of the polypeptide may differ slightly from that determined by electrophoresis. Nevertheless, it seems that endothelial cells lack the antigen recognized by IIG10 antibody, since no bands were detected on an autoradiogram of the SDS gel where the sample containing immunoprecipitate of endothelial cell Triton X-100 extract was run (fig.3). Thus, the antibody described in this paper recognizes an antigen of molecular mass 330 kDa localized on the surface of aorta smooth muscle cells and fibroblasts. The antigen was not detected in the endothelial cells. Distribution of the antigen in other tissues and cells has yet to be studied. But even now it is clear that antibody IIG10 exhibiting specificity demonstrated in these studies provides a good vector for the directed transport of substances to target (vascular smooth muscle) cells.

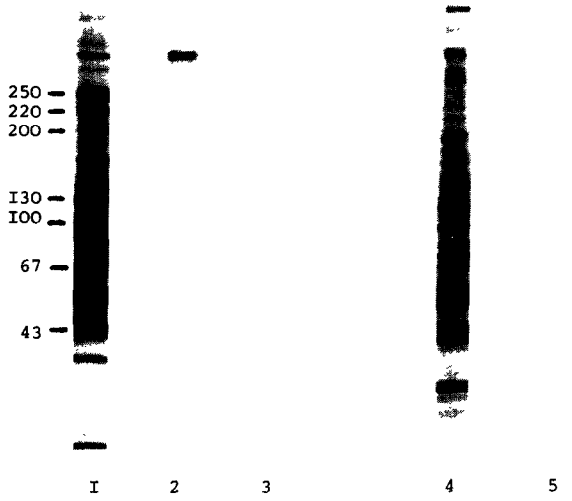

Fig.3. Autoradiograph of the SDS-polyacrylamide gel (5–15%) depicting ^{35}S-labeled proteins in Triton X-100 extracts and immunoprecipitates. (1) Triton X-100 extract of human aorta smooth muscle cells; (2) immunoprecipitate, obtained from the extract of smooth muscle cells with the antibody IIG10; (3) the same as (2), but nonimmune mouse immunoglobulins were added instead of the primary antibody; (4) Triton X-100 extract of human aorta endothelial cells; (5) immunoprecipitate, obtained from the extract of endothelial cells with the antibody IIG10.

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