Monoclonal antibody to desmin purified from cow Purkinje fibers reveals a cell-type specific determinant

I. Virtanen[1], O. Närvänen[1] and L.-E. Thornell[2]

[1]*Department of Anatomy, University of Helsinki, Siltavuorengenger 20, SF-00170 Helsinki, Finland* and [2]*Department of Anatomy, University of Umeå, Umeå, Sweden*

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We have raised monoclonal antibodies (Mab) to the M_r 55000 desmin polypeptide, electrophoretically purified from cytoskeletal preparations of isolated bovine heart Purkinje fibers One of the Mabs, 39AB6, revealed desmin only in cow Purkinje fibers and did not react with desmins from other muscle cells, including ventricular cardiac muscle, striated muscle and smooth muscle, as revealed by both immunoblotting and immunocytochemistry Desphosphorylation of electrophoretically separated polypeptides on nitrocellulose with alkaline phosphatase did not affect the binding of the Mab The present results show that there are cell-type specific antigenic determinants in intermediate proteins of the desmin type

Cytoskeleton, Desmin, Intermediate filament, Purkinje fiber

1. INTRODUCTION

Intermediate filaments (IF) are cytoskeletal proteins found thus far in most nucleated cells [1–3]. Different major cell types in mammalian tissues express distinct IF-forming proteins: vimentin in mesenchymal cells, desmin in muscle cells and specific IF proteins exist also for glial, epithelial and neuronal cells [1–3].

Desmin is an M_r 55 000 polypeptide, found thus far only in various muscle cells, including striated, cardiac and most smooth muscle cells [1,3–5]. We have recently shown that in the Purkinje fibers of cow heart, desmin shows at least 5 major isoelectric variants in 2-D gel electrophoresis, apparently due to differences in the phosphorylation status [6,7].

Here we show by using a new Mab, 39AB6, that there are cell specific determinants in desmin in cow tissues as only desmin from Purkinje fibers was recognized with the Mab.

2. MATERIALS AND METHODS

2.1. *Tissue preparation and protein purification*

Isolated Purkinje fibers [8] and ventricular myocardium of bovine heart, bovine gastric smooth muscle and striated muscle were collected and were either fixed for immunohistochemical studies or treated with Triton X-100, followed by high and low ionic strenght KCl solutions and with KI [9]. The pellets were solubilized in the electrophoresis sample buffer and subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [10], by using 8% slab gels under reducing conditions. Desmin was purified from the gels by electroelution.

Correspondence address: I. Virtanen, Department of Anatomy, University of Helsinki, Siltavuorenpenger 20, SF-00170 Helsinki, Finland

2.2. *Immunological procedures*

The Mab production was done by using standard procedures [11], as described in [12]. Briefly, Balb/c mice were immunized with ca. 50 μg of purified desmin from cow Purkinje fibers, 3 times intradermally. X63-Ag 8.653 myeloma cells were used for cell fusion Cloning was done manually [12]. The hybridomas were tested in an enzyme-linked immunoassay (EIA) by using as an antigen desmins purified by electroelution from different tissues, as well as frozen sections and cultured rhabdomyosarcoma cells as targets in immunofluorescence assay. One of the hybridomas, 39AB6, was taken under closer examination. The Mab was of the IgM class, as judged by the radial immunodiffusion technique with rabbit anti-mouse Ig-isotypes (Sigma, St. Louis, MO). Another monoclonal antibody, 37EH11, detecting desmin in all types of muscle cells [13] was applied for control purposes. For immunohistochemical staining, the sections, cut at 5 μm, were exposed to the hybridoma culture media, followed by the avidin-biotin detection method (Vector Laboratories, Burlingame, CA).

For the immunoblotting technique, the SDS-PAGE separated polypeptides were transferred onto nitrocellulose sheets [14] and were either stained for proteins or exposed to the Mabs, followed by a peroxidase-coupled swine anti-mouse Ig antiserum (Dakopatts, Glostrup, Denmark). In a set of experiments, the nitrocellulose sheets were pretreated with alkaline phosphatase (Sigma) to dephosphorylate the polypeptides (cf. [7]).

3. RESULTS

Immunocytochemical staining of frozen sections of bovine heart revealed that the Mab 37EH11 reacted with Purkinje cells, cardiac muscle cells and smooth muscle cells (Fig. 1a), while the Mab 39AB6 only reacted with the large Purkinje cells (Fig. 1b).

In immunoblotting of SDS-PAGE-separated cytoskeletal polypeptides, the Mab 37EH11 reacted with a prominent M_r 55 000 polypeptide in Purkinje fibers (Fig. 2, lane 2), smooth muscle cells (Fig. 2, lane 5), striated muscle cells (Fig. 2, lane 8) and ventricular car-

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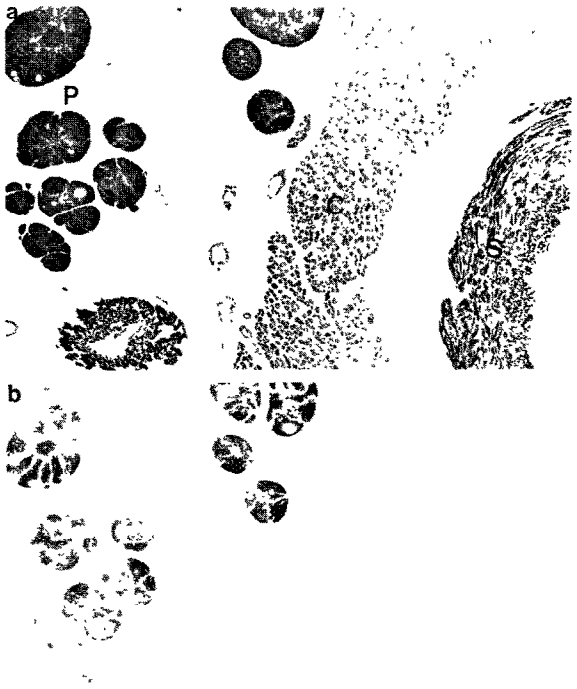

Fig. 1. Immunoperoxidase staining of consecutive sections of cow heart with Mabs 37EH11 (a) or 39AB6 (b). Note, that both Purkinje cells (P), ventricular cardiac muscle cells (C) and smooth muscle cells (S) show reaction in (a), whereas only the Purkinje cells show immunoreaction in (b). ×150.

diac muscle cells (Fig. 2, lane 11). The Mab 39AB6 reacted with the M_r 55 000 polypeptide in Purkinje fibers (Fig. 2, lane 3) but did not react with desmins of other muscle cells (Fig. 2, lanes 6, 9 and 12).

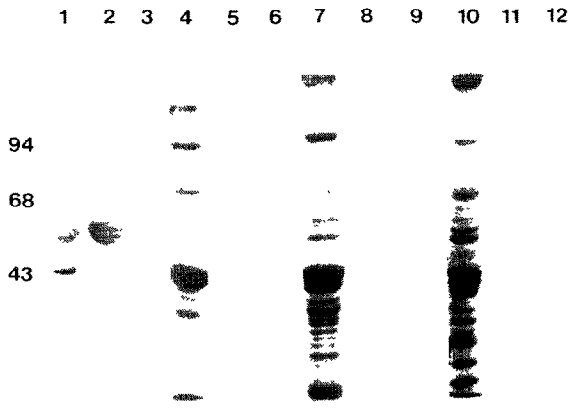

Fig. 2. Immunoblotting of electrophoretically separated cytoskeletal proteins of Purkinje fibers (lanes 1–3), smooth muscle (lanes 4–6), striated muscle (lanes 7–9) and cardiac muscle (lanes 10–12) with the Mabs 39AB6 (lanes 2, 5, 8 and 11) or 37EH11 (lanes 3, 6, 9 and 12). Lanes 1, 4, 7 and 10 show the corresponding protein profiles with Amido black. Note, that the Mab 39AB6 reacts with the M_r 55 000 desmin polypeptide only in Purkinje fibers whereas the Mab 37EH11 reacts with all muscle cell types. The relative electrophoretic mobilities of the standard proteins (10^3 Da) are indicated on the left.

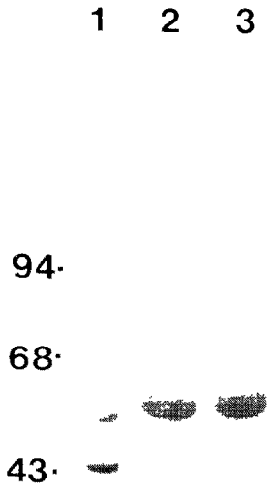

Fig. 3. Immunoblotting of electrophoretically separated polypeptides of cow Purkinje fibers with the Mab 39AB6 without (lane 2) and with treatment with alkaline phosphatase (lane 3). Similar immunoreactions with an M_r 55 000 polypeptide are revealed. Lane 1, shows the correspondig protein profile with Amido black.

Dephosphorylation of the electrophoretically transferred polypeptides with alkaline phosphatase did not have any apparent effect on the binding of the Mab 39AB6 to the M_r 55 000 polypeptide (Fig. 3, lanes 2 and 3).

4. DISCUSSION

The present study shows that in cow tissues there are cell-type specific determinants in Purkinje cell desmin that can be specifically revealed by a Mab. Although Purkinje cell desmin has been shown to differ by its many phosphorylation-dependent variants from desmins in other muscle cells [7] the present results did not suggest that the phosphorylation-status would be the basis for the Mab 39AB6-reactivity.

Earlier studies with Mabs have suggested that although a set of Mabs against desmin can distinguish some variations in immunoreactivities between different desmin-containing cells and tissues, they did not suggest any distinct immunoreactive variants. Such variants have, however, been found earlier for vimentin [7,16] and neurofilament proteins [17]. As all the desmins appear to be products of a single gene and mRNA [18,19], it remains to be elucidated what is the functional basis for a distict immunological desmin isoform in Purkinje cells.

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